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ANTIHYPERTENSIVE ACTIVITY IN RATS OF SQ 14,225, AN ORALLY ACTIVE INHIBITOR OF ANGIOTENSIN I-CONVERTING ENZYME[1]

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ABSTRACT

LAFFAN, ROBERT J., MORTON E. GOLDBERG, JOHN P. HIGH, THOMAS R. SCHAEFFER, MARGARET H. WAUGH AND BERNARD RUBIN: Antihypertensive activity in rats of SQ 14,225, an orally active inhibitor of angiotensin I-converting enzyme. J. Pharmacol. Exp. Ther. 204: 281–288, 1978.

SQ 14,225 (D-3-mercapto-2-methylpropanoyl-L-proline) markedly lowered the blood pressure of the renin-dependent aortic-ligated and two-kidney Goldblatt hypertensive rat and failed to reduce blood pressure in the one-kidney Goldblatt hypertensive rat. In the two-kidney Goldblatt rat, SQ 14,225 (p.o.) was about 10 times as potent as teprotide, the nonapeptide SQ 20,881 (s.c.). Oral doses of SQ 14,225 moderately reduced the blood pressure of the Wistar-Kyoto spontaneously hypertensive rat but not that of the normotensive Wistar-Kyoto rat. Bilateral nephrectomy abolished the antihypertensive activity of SQ 14,225 in the spontaneously hypertensive rat. SQ 14,225 and SQ 20,881 elicited parallel dose-response curves in the two-kidney renal hypertensive rat. Post-treatment of spontaneously hypertensive rats with either agent failed to augment the antihypertensive effect produced by effective doses of the other agent. The results suggest that SQ 14,225 acts primarily by inhibiting the renin-angiotensin system to reduce elevated blood pressure, especially in presumably renin-dependent models of hypertension.

Recent developments of understanding the renin-angiotensin-aldosterone system have been very instrumental in advancing our knowledge of clinical hypertensive states. Laragh *et al.* (1977) have discussed the effect of modification of this system by use of specific inhibitors on the vasoconstrictor and volume abnormalities in this disease state. They have speculated on the possible implications of advances in this field on the classification and treatment of patients with renovascular or essential hypertension. The need for additional specific agents which inhibit angiotensin I-converting enzyme (ACE), a peptidyldipeptide hydrolase, in hypertensive subjects is implicit to their discussion. Furthermore, since such inhibitors may also inhibit the inactivation of bradykinin, part of the antihypertensive action of such agents may also be attributable to

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effects on the kallikrein-kinin-prostaglandin system (Erdös, 1976; McGiff and Nasjletti, 1976; Hollenberg and Williams, 1978).

SQ 14,225 (D-3-mercapto-2-methylpropanoyl-L-proline), designed as a novel inhibitor of ACE, based on a hypothetical model of the active site of this enzyme, has been reported to be a potent and specific orally active inhibitor of this enzyme by Ondetti *et al.* (1977) and Rubin *et al.* (1977). They found that the compound lowered the blood pressure in selected models of experimental hypertension. In this report, we have confirmed these results and have investigated further both the spectrum of oral and parenteral activity of SQ 14,225 in other models of experimental rat hypertension and the relationship of this agent to the nonapeptide inhibitor of ACE, SQ 20,881. In contrast to SQ 14,225, the antihypertensive efficacy of SQ 20,881 is limited to parenteral use in both animals and in man (Engel *et al.*, 1973; Laragh *et al.*, 1977; Rubin *et al.*, 1977; Ferguson *et al.*, 1977).

Methods

Anesthetized rats. An accelerated severe renal hypertension was induced in male Sprague-Dawley rats (250 ± 30 g) by complete ligation of the aorta under ether anesthesia midway between the origin of the renal arteries as described by Carretero *et al.* (1971). Four to 7 days later, the hypertensive rats were anesthetized with urethane (1.6 g/kg) i.m. and injected i.v. with atropine sulfate (1 mg/kg). Carotid arterial blood pressure was monitored for at least 2 hours using a Statham P23Gb transducer in series with a Beckman Dynograph recorder. These rats each received i.v. infusions of aqueous solutions of SQ 14,225, 0.3 or 1.0 mg/kg/min, or physiological saline for 10 minutes at a rate of 0.1 ml/min.

Unanesthetized rats. *Recording.* Indwelling abdominal aortic catheters were implanted in rats anesthetized with pentobarbital Na, according to the method of Weeks and Jones (1960). At least 1 week later their direct blood pressure and heart rate were recorded by the method of Laffan *et al.* (1972) modified as follows. The signal from the transducer was digitized in a 10-bit analog to digital converter and input to a PDP 11/05 computer. The computer was programmed to sense and store samples at a rate of 125/sec for each rat, as well as the number of pressure pulses during 10 seconds of each scan on each rat. These parameters were averaged and stored as the mean blood pressure, (MBP, millimeters of mercury) and heart rate (beats per minute) for that time. Data were acquired from each rat every 5 minutes. Six such sets of data were averaged to give a mean value representing a 30-minute sample and this 30-minute figure was stored for subsequent analysis. Each time a 48-hour cycle was completed (or sooner if demanded) the data were transferred serially to a host computer (PDP 11/40) for further analysis and were printed out on a Versatec printer/plotter for at least 16 hours after each dose.

Renal hypertensive rats. Male rats (120–150 g) of the Charles River CFN Wistar strain were anesthetized with ether and a silver clip (0.22 mm inside diameter) was placed on the left renal artery through a flank incision. The contralateral kidney was either left intact (two-kidney Goldblatt model: 2-K RHR) or excised (one-kidney Goldblatt model: 1-K RHR). Five weeks later the animals were intubated according to the method of Weeks and Jones (1960) to prepare them for blood pressure and heart rate determination during the subsequent week by the method described above.

Spontaneously hypertensive rats. Ten to 14-week-old male Wistar-Kyoto normotensive rats (220 ± 50 g) and spontaneously hypertensive rats (230 ± 50 g) of the Wistar-Kyoto Okamoto-Aoki strain hereinafter identified as NR or SHR, respectively, were obtained from Taconic Farms, Germantown, N.Y. They were given food and water *ad libitum* and intubated as described above.

In other studies the SHR were subjected to bilateral nephrectomy under ether anesthesia and their blood pressure and heart rate responses to the test compound were determined as described above, 18 hours after surgery.

Experimental procedures and statistics. In most studies, except where noted, SQ 14,225 was given orally and SQ 20,881 subcutaneously to separate groups of rats for 2 consecutive days; 24 hours elapsed between doses. Unless otherwise noted, all graphical and text data show means ± S.E.; in some figures 1 S.E. is shown either above or below the mean to improve clarity. Log $dose_{10}$-response curves and linear regression analyses were calculated by the least-squares method.

SQ 14,225 and the nonapeptide SQ 20,881 were synthesized at the Squibb Institute for Medical Research, Princeton, N.J.

Results

Intravenous infusion of saline to anesthetized aortic-ligated rats did not initially decrease either the systolic or diastolic blood pressure; however, a slight reduction in these values was seen over the ensuing 2 hours (figure 1). In contrast, infusion with a total of either 3 or 10 mg/kg of SQ 14,225 over a 10-minute period produced a mild, transient decrease in systolic

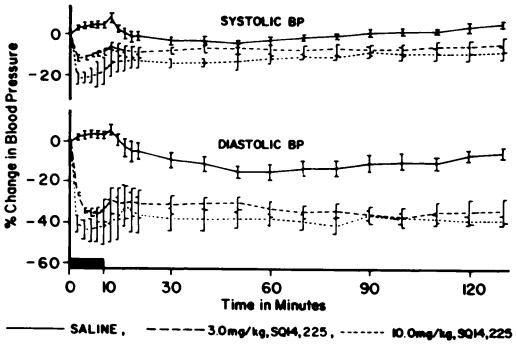

FIG. 1. Antihypertensive effects of a 10-minute i.v. infusion of SQ 14,225 in urethane-anesthetized aortic-ligated rats. Doses of SQ 14,225 are total doses of SQ 14,225 infused. Initial systolic blood pressure (BP) ranged from 158 ± 5 to 163 ± 5 mm Hg; initial diastolic blood pressure ranged from 106 ± 3 to 117 ± 7 mm Hg. Three to five rats were used for each treatment. The mean systolic BP ± S.E. and the mean diastolic BP ± S.E. are shown in the upper and lower sections, respectively.

pressure and a marked, sustained decrease in diastolic blood pressure of 36 to 45% (to levels of 69 and 65 mm Hg, respectively) during the 2-hour test period.

SQ 14,225 was administered by gavage to conscious 2-K RHR at 1, 3, 10 or 30 mg/kg/day and to 1-K RHR at 3 or 30 mg/kg/day whereas NR were given 3, 10, 30 or 100 mg/kg/day and SHR were given 0.3, 1, 3, 10, 30 or 100 mg/kg/day. Saline controls were studied with each type of conscious rat used and saline was given at a dose of 5 ml/kg/day. Two of the dose levels shared by all groups, namely 3 and 30 mg/kg/day, were selected for purposes of brevity and clarity, along with a saline control group for graphic presentation in figures 2 through 4. As shown in figure 2, SQ 14,225 produced a marked prompt and sustained antihypertensive effect in the 2-K RHR. Furthermore, with the higher dose of 30 mg/kg p.o., the effect persisted such that the blood pressure had not returned to control levels when the second dose was administered 24 hours later. However, the blood pressure fell to about the same nadir after this dose as that observed on the 1st day. These same doses did not significantly alter the blood pressure of the 1-K RHR after the first dose (fig. 3) or after a second dose given on the 2nd day (data not shown).

A dose-related antihypertensive effect was also seen in the SHR model. As with the 2-K RHR, the effect of SQ 14,225 was more prolonged with higher doses and a significant residual drug effect was observed at the time of the second administration of SQ 14,225 (fig. 4). Groups of NR failed to exhibit a significant dose-related hypotensive effect when given SQ 14,225 in doses from 3 to 100 mg/kg/day for 2 consecutive days. A summary of these data for NR as well as a graphic comparison of the dose-maximum antihypertensive effects of all oral doses of SQ 14,225 administered to the 2-K RHR and SHR on the 1st test day is shown in figure 5. As seen in this figure, there was (1) no linear regression in the NR model, (2) significant linear regression in the SHR model ($P < .01$) and (3) significant linear regression in the 2-K RHR model ($P < .001$). The slope of the 2-K RHR was significantly steeper ($P < .01$) than that in the SHR model.

A summary of the effects of SQ 14,225 on the heart rate of these models is presented in table 1. Tachycardia was seen in all four models (1-K RHR, 2-K RHR, NR and SHR) especially within the 1st hour after dosage. However, this occurred after both saline and SQ 14,225 administration and apparently was not related

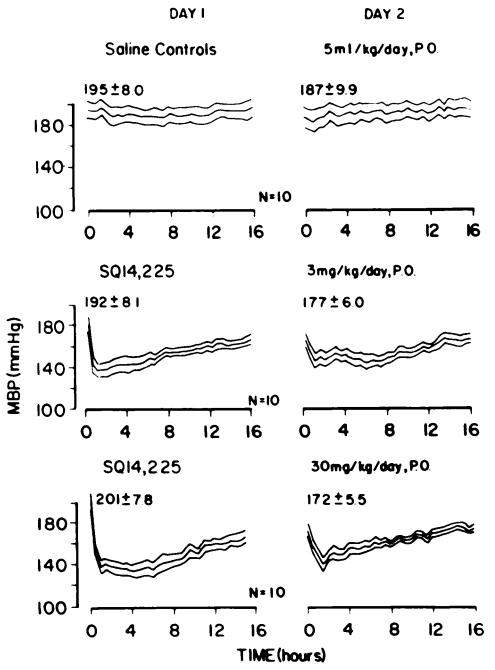

FIG. 2. Antihypertensive effects of single daily oral doses of SQ 14,225 to two groups of 10 unanesthetized 2-K RHR. A third group of 10 such rats received only saline (5 ml/kg/day) orally. The initial MBP ± S.E. (millimeters of mercury) is shown for each daily plot. The three lines in each panel refer to the MBP ± S.E.

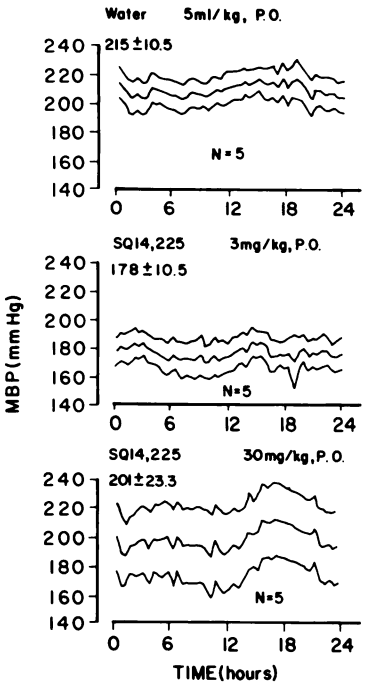

FIG. 3. Effects of MBP of single oral doses of SQ 14,225 to two groups of five unanesthetized 1-K RHR. A third group of five such rats received only water (5 ml/kg) orally. The initial MBP ± S.E. (millimeters of mercury) is shown for each plot. The three lines in each panel refer to the MBP ± S.E.

to the degree or duration of the hypotensive activity or to the dose of SQ 14,225.

The nonapeptide, SQ 20,881, exhibited antihypertensive activity in the 2-K RHR but not in the 1-K RHR after subcutaneous doses exceeding 10 mg/kg/day. A graphic comparison of the dose-maximum antihypertensive effects of subcutaneously administered SQ 20,881 and orally administered SQ 14,225 in the 2-K RHR on the 1st test day is presented in figure 6. Parallel dose-response curves were obtained; SQ 20,881 was about $^1/_{10}$ as potent as SQ 14,225 in 2-K RHR.

In the SHR model, SQ 20,881 and SQ 14,225 produced equivalent antihypertensive responses. The data presented in figure 7 show that during the first 4 hours after drug administration, the response of SHR to oral administration of 30 mg/kg of SQ 14,225 was indistinguishable from the blood pressure decrease seen after the same dose of SQ 20,881 given subcutaneously. Subsequent administration of the same dose of either inhibitor of ACE in crossover fashion did not further reduce the blood pressure or affect the course of blood pressure recovery.

SQ 14,225 was further tested by administering the compound to nephrectomized SHR. Nephrectomy did not change resting blood pressure; however, it reduced the hypotensive response of 3 mg/kg p.o. of SQ 14,225 (fig. 8) previously observed in intact SHR. None of these treatments significantly affected the heart rates of these animals.

To rule out the possibility that tolerance might develop to the subacute administration of SQ 14,225 in SHR, groups of rats were given either 3 or 30 mg/kg/day of SQ 14,225 for 11 consecutive days. The data from the 1st and 11th day of dosage are presented in figure 9. No reduction or attenuation of the antihypertensive response to SQ 14,225 was noted. Instead, the effect was maintained for a slightly longer time after repeated administration. MBP at the start of the study was approximately 190 mm Hg. Twenty-four hours after the rats received the 10th daily dose of SQ

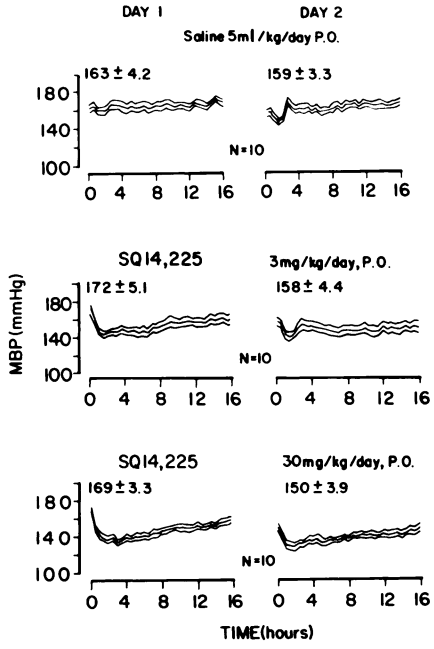

FIG. 4. Effects on MBP of single oral daily doses of SQ 14,225 to two groups of 10 unanesthetized Wistar-Kyoto SHR. A third group of 10 such rats received only saline (5 ml/kg/day) orally. The initial MBP ± S.E. (millimeters of mercury) is shown for each daily plot. The three lines in each panel refer to the MBP ± S.E.

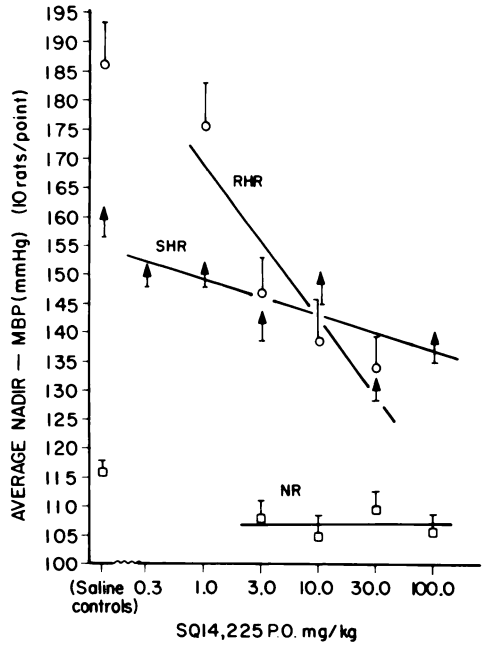

FIG. 5. Comparison of maximum antihypertensive effects of single oral doses of SQ 14,225 to unanesthetized 2-K RHR (○), Wistar-Kyoto SHR (▲), and Wistar-Kyoto NR (□). Linear regression equations: RHR, $y = 168.72 - 26.77x$, $r = -0.594$, $P < .001$; SHR, $y = 149.02 - 6.37x$, $r = -0.404$, $P < .01$; NR, $y = 107.56 - 0.23x$, $r = -0.016$, $P > .80$. The MBP + or − S.E. is shown on each of the three lines.

14,225 (3 or 30 mg/kg/day), the blood pressure in each group was approximately 165 mm Hg ($p < .05$). However, the percent reduction in pressure during the 1st hour after dosage with SQ 14,225 on the 11th day was not significantly different from that obtained on the 1st day.

Discussion

The antihypertensive response to SQ 14,225 administration in the urethane-anesthetized aortic-ligated rat and in the 2-K RHR confirms and extends the observations of Ondetti *et al.* (1977). This agent inhibits the conversion of angiotensin I to angiotensin II, inhibits the inactivation of bradykinin and lowers blood pressure in presumably renin-dependent models of hypertension. Furthermore, the absence of antihypertensive efficacy in the conscious 1-K RHR model after 2 days of p.o. dosage with SQ 14,225, is in agreement with the above. Koletsky *et al.* (1967, 1971) have shown a renin-dependent hypertension in the aortic-ligated rat while several groups have shown that the renin-angiotensin system is involved in the pathogenesis of the 2-K RHR but not of the 1-K RHR model (Brunner *et al.*, 1971; Doyle *et al.*, 1976; Swales *et al.*, 1971).

Findings similar to those obtained in this

TABLE 1

Effect of oral doses of SQ 14,225 on heart rate of rats

Model	Oral Dose	Heart Rate at Time on Day 1			
		0 hr	0.5 hr	1.0 hr	8.0 hr
	/kg		*beats/min*		
1-K RHR[a]	H_2O, 5 ml	399.0 ± 4.0	428.0 ± 7.2[b]	412.0 ± 6.4	389.0 ± 11.0
	3.0 mg	397.0 ± 13.3	432.0 ± 10.8	399.0 ± 16.0	406.0 ± 13.0
	30.0 mg	410.0 ± 20.2	441.3 ± 12.3	427.5 ± 10.9	405.0 ± 7.4
2-K RHR[c]	Saline, 5 ml	376.5 ± 12.2	387.5 ± 9.9	376.5 ± 12.7	376.0 ± 10.7
	3.0 mg	380.0 ± 8.6	414.0 ± 9.4[b]	404.0 ± 10.1	369.5 ± 10.7
	30.0 mg	388.0 ± 11.1	434.0 ± 10.1[b]	418.0 ± 11.9	369.0 ± 12.4
NR[c]	Saline, 5 ml	353.5 ± 6.7	414.5 ± 6.9[b]	387.0 ± 5.1[b]	346.5 ± 6.2
	3.0 mg	345.0 ± 13.1	427.5 ± 7.6[b]	388.5 ± 7.5	375.5 ± 5.2
	30.0 mg	339.5 ± 12.5	401.5 ± 12.3[b]	405.0 ± 13.1[b]	383.0 ± 7.0[b]
SHR[c]	Saline, 5 ml	366.0 ± 12.1	401.5 ± 7.7[b]	379.9 ± 9.5	362.0 ± 7.3
	3.0 mg	368.0 ± 9.7	413.0 ± 114[b]	396.5 ± 10.4	364.5 ± 9.1
	30.0 mg	365.0 ± 13.9	407.0 ± 14.8	397.5 ± 11.6	360.5 ± 4.4

[a] $N = 5$ rats/group.
[b] $P < .05$, paired t test.
[c] $N = 10$ rats/group.

report were observed by Engel *et al.* (1973) who used the nonapeptide SQ 20,881 to block angiotensin-converting enzyme. Thus, two available angiotensin-converting enzyme inhibitors of diverse chemical structures lower MBP in those hypertensive states which appear to be largely renin-dependent for maintenance of elevated blood pressure. When these two agents, SQ 20,881 and SQ 14,225, were compared directly in the 2-K RHR model, the resulting dose-maximum response curves were parallel, suggesting a common mechanism(s) of action.

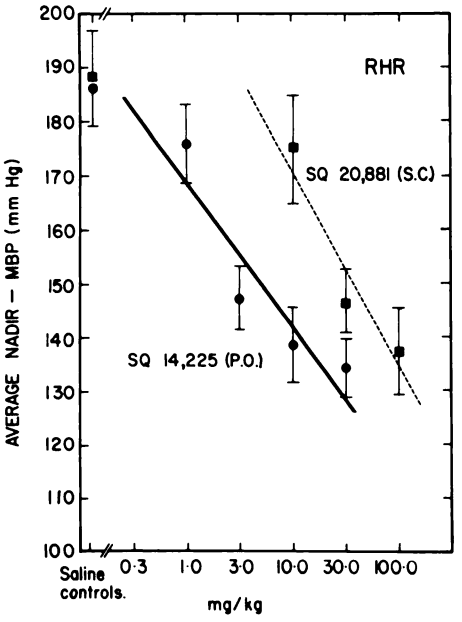

FIG. 6. Dose-antihypertensive effects of SQ 20,881, five rats per dose in 6 weeks 2-K RHR. Linear regression equation for s.c. SQ 20,881 (■): $y = 208.24 - 36.88x$, $r = -0.651$, $P < .01$. The data for p.o. SQ 14,225 (●) are identical to those shown in figure 5. The two dose-response curves do not depart from parallelism ($P > .40$). The MBP ± S.E. is shown for each line.

The involvement of the renin-angiotensin system in the pathogenesis and maintenance of the blood pressure of the SHR is controversial. Plasma renin activity and concentration have been reported to be high, near normal or suppressed (DeJong *et al.*, 1972; Koletsky *et al.*, 1970; Sen *et al.*, 1972; Barrett *et al.*, 1976). Campbell (1974) and Barrett *et al.* (1976), while finding these parameters to be normal, have indicated that this animal model, as well as the 2-K RHR model, has elevated renin substrate levels. Thus, whatever the mechanism, a dependence of blood pressure of the SHR on the renin angiotensin system, resulting from renin levels inappropriately high for the level of sodium balance, seems to be a distinct possibility. The results obtained in the present study indicate that SQ 14,225, a specific inhibitor of angiotensin-converting enzyme, lowers the blood pressure in SHR. This compound does not appear to possess adrenergic agonist or antagonist properties, ganglion-blocking activity, smooth muscle relaxant activity, peripheral vasodilator activity or the ability to lower

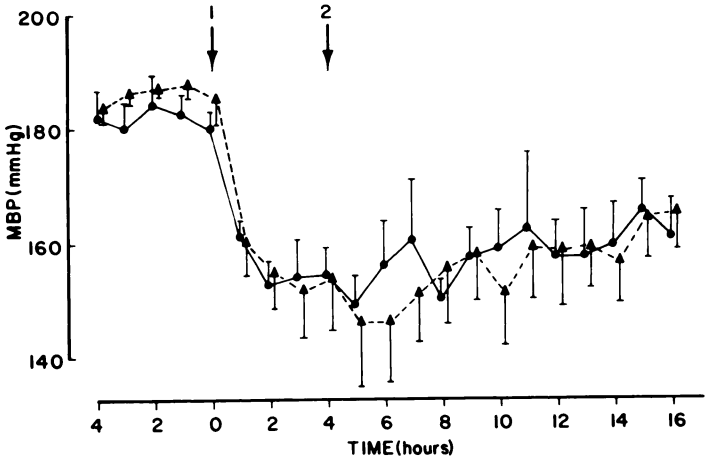

FIG. 7. Lack of interaction of SQ 14,225 and SQ 20,881 on MBP (millimeters of mercury) in unanesthetized Wistar-Kyoto SHR. At the point indicated by arrow marked 1, the group represented by ▲ received an oral dose of 30 mg/kg of SQ 14,225 while the group represented by ● received 30 mg/kg of SQ 20,881 subcutaneously. At the arrow marked 2 the compounds were crossed over. (See "Methods" for more details.) $N = 5$/group. The MBP + or − S.E. is shown for each line.

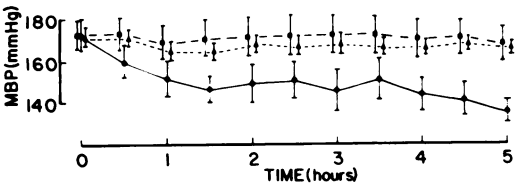

FIG. 8. Effect of SQ 14,225 on MBP (millimeters of mercury) of unanesthetized, nephrectomized, Wistar-Kyoto SHR. ●, 3 mg/kg p.o. in intact SHR; ▲, 3 mg/kg p.o. in nephrectomized SHR; ■, 5 ml of water per kg p.o. in nephrectomized SHR. N = 5/group. The MBP ± S.E. is shown for each line.

blood pressure when injected centrally (Rubin *et al.*, 1977; Boccagno and Vollmer, 1977; R. R. Vollmer and V. S. Murthy, unpublished observations). Thus, its antihypertensive properties appear to be related to consequences resulting from specific inhibition of this enzyme. The slope of its dose-response curve in SHR is clearly flatter than that obtained in 2-K RHR models. This difference might indicate that the renin-angiotensin system plays a lesser but substantial role in sustaining the blood pressure in SHR. It is conceivable, however, that rat strain differences may exaggerate this divergence. Until we have contrasted the effects of SQ 14,225 in NR and in SHR rendered hypertensive or further hypertensive in a similar way as in 2-K RHR, this remains a tentative conclusion only.

SQ 14,225 did not lower the blood pressure of nephrectomized SHR. This may offer support to renin angiotensin involvement although the renal kallikrein-kinin system may also be involved. It does, however, tend to exclude a major role for participation of extrarenal kinins in the antihypertensive response to this agent. Eaton and Poisner (1977) have shown that plasma renin levels of nephrectomized Wistar rats were reduced to negligible levels. Of interest are the clinical results of Case *et al.* (1976) who have shown that SQ 20,881 was devoid of antihypertensive activity in anephric patients. It would appear, regardless of mechanism, that the kidneys plan an important and permissive role in expression of the antihypertensive effects of inhibitors of ACE. Further and more complete dose-response relationships are required beyond this preliminary one-dose study in nephrectomized rats. Our results do suggest that SQ 14,225 and SQ 20,881 appear to act as antihypertensive agents through common mechanism(s) as suggested by parallel dose-response curves in 2-K RHR and failure of either agent to further reduce blood pressure after pretreatment with the other inhibitor. Conceivably other types of antihypertensive agents could have further reduced blood pressure below the maximal 45 mm Hg decrease (fig. 7) obtained after inhibition of ACE in SHR.

The present studies indicate that SQ 14,225 produced no evidence of reduced effectiveness after 11 days of treatment in reducing blood pressure of SHR. Additional studies in progress, in both 2-K RHR and SHR, have now extended daily treatment with SQ 14,225 well beyond 1 month; no tolerance to its antihypertensive properties has been noted (B. Rubin, unpublished observations).

The specificity of SQ 14,225 as an inhibitor of the peptidyldipeptide hydrolase ACE (EC 3.4.15.1), demonstrated by Rubin *et al.* (1977), would indicate that inhibition of angiotensin II synthesis and/or augmentation of the activity of bradykinin may be involved in the antihypertensive action of this agent. Although the

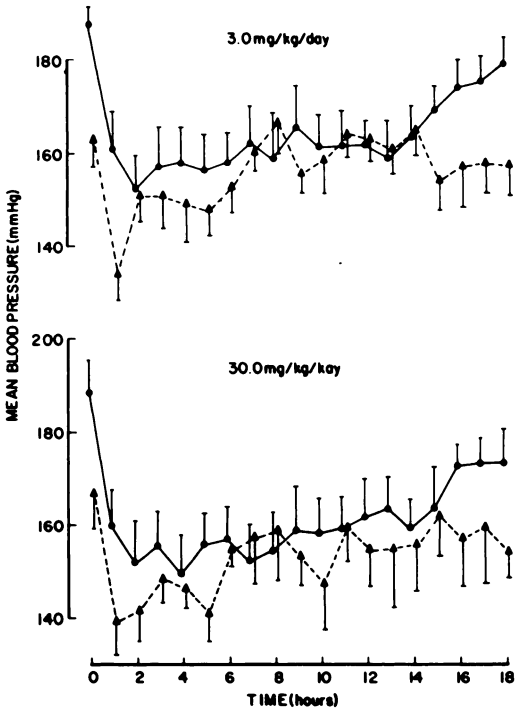

FIG. 9. Effect of repeated p.o. daily doses of 3 or 30 mg/kg of SQ 14,225 on MBP (millimeters of mercury) in unanesthetized Wistar-Kyoto SHR. ●, first day of dosing; ▲, last day of dosing, following 10 previous daily doses. N = 5 or 6/group. The MBP + or − S.E. is shown for each line.

contributions of renal kinins, prostaglandin (or other autocoids) in the activity of SQ 14,225 must await further research, it is probable that this agent effectively reduces blood pressure in presumably renin-dependent models of rodent hypertension as a result of inhibition of this enzyme.

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