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Effects of the Oral Converting Enzyme Inhibitor (SQ 14225) on One-Kidney Hypertension in the Dog (40030)

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In two recent preliminary studies (1, 2), it was demonstrated that chronic one-kidney hypertension developed in the dog despite continuous angiotensin blockade during the acute high renin phase of this experimental disease. The angiotensin II antagonist, [Sar^1, Ala^8] angiotensin II, and the converting enzyme inhibitor, SQ 20881, were given continuously for 1 and 2 days before and for 6 and 7 days after renal artery constriction. During [Sar^1, Ala^8] angiotensin II infusion (1), arterial pressure appeared to increase during days 2-4 after renal artery constriction but the change was not significantly different from the level of arterial pressure during analog infusion in normal control dogs until the fifth day after renal artery stenosis. At this time, the level of arterial pressure achieved was characteristic of the chronic hypertensive state. During SQ 20881 infusion (2), arterial pressure was not significantly elevated until the sixth day after renal artery constriction. This delay in the development of hypertension during SQ 20881 infusion appeared to result from angiotensin blockade; also, a possible concurrent rise in the plasma level of bradykinin and the associated depressor influence of this peptide was considered. As soon as the SQ 20881 infusion was discontinued arterial pressure increased further and the chronic hypertensive state was achieved. The findings demonstrate that the renin-angiotensin system need never be activated for the development and maintenance of chronic renovascular hypertension.

In the present study, the new oral converting enzyme inhibitor, SQ 14225, was given to dogs 1 day before and 7 days after renal artery stenosis to evaluate further the effects of converting enzyme inhibition. This enzyme converts angiotensin I to angiotensin II and degrades bradykinin. SQ 14225 given orally to rats inhibited the pressor effect of angiotensin I, augmented the depressor action of bradykinin and lowered arterial pressure in experimental renin-dependent renovascular hypertension (3); also McCaa (4) has a preliminary report to show that SQ 14225 lowered arterial pressure and plasma aldosterone concentration and increased plasma renin activity (PR) in conscious sodium-deficient dogs. The purpose of the present study was to determine the efficacy of orally administered SQ 14225 on the development of experimental one-kidney hypertension in dogs and to compare this response to that obtained earlier (1, 2) with [Sar^1, Ala^8] angiotensin II and SQ 20881.

Methods. Three female hounds (15-19 kg) were unilaterally nephrectomized under sterile conditions and catheters were placed in a femoral artery and vein for chronic studies; the catheters were exteriorized between the shoulder blades. The animals were allowed to recover for several days and they were trained to lie quietly on a table. Daily measurements of arterial pressure and heart rate were made each morning between 8:00-8:30 with a Statham pressure transducer and a Sanborn recording system, and blood was drawn for determination of plasma electrolytes, PRA and plasma aldosterone concentration. The blood samples were collected in tubes containing 0.1 ml of 10% ethylene-diaminetetraacetic acid (EDTA) per 10 ml of blood; the samples were cooled in an ice bath and centrifuged in cold. Plasma was stored frozen until time of radioimmunoassay for PRA and aldosterone. Plasma was prepared for generation of angiotensin I by dialyzing it against a phosphate buffer (pH 5.4) for 18 hr (three changes); diisopropylflourophosphate (DFP) and sodium chloride were added to the plasma prior to incubation at 37° for 60 min. The reaction was then stopped by

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placing the tubes in a bath of ice-water. Angiotensin I content was quantified by radioimmunoassay (5) and PRA is expressed as nanograms of angiotensin I/ml/hr. Plasma aldosterone concentration was determined by the method of Bühler, Sealey and Laragh (6); aldosterone was separated by celite column chromatography and quantified by radioimmunoassay. The dogs were fed a daily diet containing 60–65 mEq sodium and were housed in metabolic cages for the determination of sodium balance.

After measurements were made for two days, the converting enzyme inhibitor SQ 14225 (D-2-methyl-3-mercaptopropanoyl-L-proline) was given orally for 8 days in divided doses at 8:30 AM, 4:30, and 10:30 PM. The efficacy of SQ 14225 in producing blockade of angiotensin I was assessed daily before the morning dose by determining the pressor response to 2 and 4 μg of intra-arterial injections of exogenous angiotensin I; the peptide was given intra-arterially rather than intravenously because an indwelling arterial catheter was available. On the second day of SQ 14225 administration, the renal artery was constricted to reduce renal blood flow by 55–60%, a stenosis sufficient to produce benign one-kidney renal hypertension in dogs within 24 hr. (7). Administration of SQ 14225 was continued for 7 days after renal artery constriction to encompass the entire early high renin phase of renal hypertension and measurements were continued. Observations were made during a 5-day recovery period.

The first two dogs studied, received 10 mg/kg of SQ 14225 three times daily for the day before and for 5 days after renal artery constriction. Occasionally, a slight pressor response to the 4 μg dose of angiotensin I was observed. Consequently, the 10:30 PM dose of SQ 14225 was increased from 10 to 15 mg/kg for the remaining two days of angiotensin blockade. In the third dog, 15 mg/kg of SQ 14225 was given at 10:30 PM throughout the study so the total dose was 35 mg/kg/day.

Dose response curves for the depressor action of bradykinin triacetate were obtained by giving bolus intravenous injections from 0.1 to 30 μg/kg to all three dogs before and to two of the three animals on the sixth day of SQ 14225 administration.

Data from all experiments were analyzed by use of the Student's paired t test, critical to a 5% level of significance.

Results. The completeness of angiotensin blockade was demonstrated by finding only slight pressor responses to injections of angiotensin I during SQ 14225 administration. Before the inhibitor was given, 2 μg of angiotensin I increased arterial pressure by 23 ± 2.0 mm Hg while 4 μg gave a response of 32 ± 2.8 mm Hg.; during SQ 14225 administration, a response of 6.5 ± 1.5 mm Hg occurred in 13% of the 2 μg injections and a response of 7 ± 1 mm Hg occurred in 50% of the 4 μg injections.

During the first day of angiotensin blockade and before renal artery constriction mean arterial pressure decreased 9 mm Hg ($P < 0.05$) but the changes in PRA and plasma aldosterone concentration were not significant (Table I). During continued angiotensin blockade and after renal artery stenosis, arterial pressure continued to be significantly decreased on day 1 but increased slightly each day thereafter until the fifth day at which time arterial pressure was significantly elevated above the control level. Arterial pressure was sustained at this hypertensive level for the remaining 2 days of SQ 14225 administration and during the recovery period. PRA was increased significantly throughout the period of combined angiotensin blockade and renal artery stenosis and during the first recovery day. Plasma aldosterone concentration remained at the normal control level throughout the study. Sodium excretion appeared to decrease on the day following renal artery constriction but the variability in response among the three dogs was enough to prevent a significant change from occurring.

To assess the possibility of a coincidental increase in the plasma bradykinin level during SQ 14225, dose response curves to bolus injections of bradykinin triacetate were obtained before and during drug administration (Table II). A striking increase in both the magnitude and the length of the depressor responses occurred during SQ 14225 administration.

Discussion. During the last few years, substantial evidence has accumulated to

TABLE I. EFFECTS OF ORAL ADMINISTRATION OF SQ 14225 BEFORE AND DURING THE ACUTE PHASE OF RENOVASCULAR HYPERTENSION IN THE DOG

	Days	Mean arterial pressure (mmHg)	Plasma renin activity (ng-AngI/ml/hr)	Plasma aldosterone level (ng%)
	Control 1	110 ± 5		
	Control 2	106 ± 2	0.7 ± 0.2	
	Control 3	103 ± 4	1.7 ± 0.9	5.3 ± 0.3
→	1	97 ± 5*	6.4 ± 2.3	3.8 ± 0.3
SQ 14225 (30-35 mg/kg/day)	RAC			
	1	101 ± 1*	10.4 ± 3.2*	3.7 ± 0.7
	2	103 ± 2	10.9 ± 1.1*	4.5 ± 0.3
	3	115 ± 8	7.7 ± 1.1*	5.8 ± 0.7
	4	115 ± 7	6.5 ± 1.6*	4.5 ± 1.0
	5	118 ± 5*	4.3 ± 1.0*	3.5 ± 0.4
	6	116 ± 3*	5.0 ± 0.9*	3.6 ± 0.5
←	7	121 ± 3*	4.9 ± 0.4*	4.5 ± 0.3
	Recovery			
	1	121 ± 4*	3.6 ± 0.1*	5.8 ± 0
	2	124 ± 3*	3.3 ± 0.1	3.5 ± 0.4
	3	122 ± 1*	1.7 ± 0.1	6.0 ± 1.2
	4	124 ± 2*	1.8 ± 0.1	3.8 ± 0.4
	5	123 ± 6*	1.8 ± 0.6	5.6 ± 0.4

* Denotes that value is significantly different ($P < 0.05$) from the average of the last 2 control values. The values for all three functions are presented as means ± SEM.

TABLE II. EFFECTS OF INTRAVENOUS BRADYKININ ADMINISTRATION BEFORE AND DURING ADMINISTRATION OF 35 MG/KG/DAY OF SQ 14225 IN THREE DOGS

		Bradykinin triacetate (μg/kg) as a bolus injection				
		0.1	1.0	3.0	10.0	30.0
I. Prior to SQ 14225 administration						
dog 1	Δ MAP[a]	8	15	23	29	40
	Duration[b]	21	15	21	81	102
dog 2	Δ MAP	9	25	30	38	40
	Duration	6	12	36	42	204
dog 3	Δ MAP	9	19	27	35	–
	Duration	6	18	24	180	–
II. During oral SQ 14225 administration (35 mg/day)						
dog 1	Δ MAP	27	32	49	–	–
	Duration	24	108	300	–	–
dog 2	Δ MAP	29	54	–	–	–
	Duration	21	300	–	–	–

[a] Mean arterial pressure (mmHg).
[b] Duration of depressor response (sec).

show increased activity of the renin-angiotensin system during the acute phase of both one and two-kidney hypertension in several experimental animals (8). PRA is increased for 3–6 days after renal artery constriction in the unilaterally nephrectomized dog and during this time the animals show a depressor response during bolus injections or brief periods of infusion of angiotensin II antagonists and converting enzyme inhibitors (8). This fall in arterial pressure from the hypertensive level toward or to normal indicates a pathogenic role for the renin–angiotensin system. On the other hand, angiotensin blockade throughout the entire 3- to 6-day acute high renin phase of hypertension failed to prevent the development of chronic one-kidney renovascular hypertension in the dog (1, 2).

The present observations confirm the

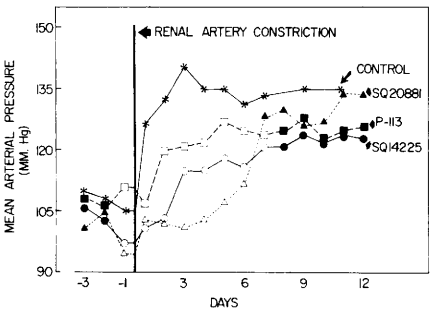

FIG. 1. Effects of angiotensin blockade on the development of one-kidney hypertension in dogs. The open symbols indicate the time of drug administration. The control group of 6 dogs was treated identical to the present experimental group receiving SQ 14225 except that no drug was given (7). The results with SQ 14225 show the present data on a dose of 35 mg/kg/day given orally to three dogs. The angiotensin antagonist, [Sar^1, Ala^8] angiotensin II (P-113) (1) and the converting enzyme inhibitor, SQ 20881, (2) were given intravenously by constant infusion at doses of 1 μg/kg/min (six dogs) and 10 μg/kg/min (five dogs) respectively.

findings reported earlier (1, 2) that the renin–angiotensin system need never be activated for the development and maintenance of chronic renal hypertension. Indeed, the blood pressure response to renal artery constriction during SQ 14225 administration was similar to that reported (1) with [Sar^1, Ala^8] angiotensin II (Fig. 1) except that the pressor response to renal artery constriction during SQ 14225 was slightly slower and somewhat less than with [Sar^1, Ala^8] angiotensin II; with both drugs, however, arterial pressure was not significantly elevated until the 5th day after renal artery stenosis. At this time and, thereafter, a chronic hypertensive state was present. The arterial pressure response during SQ 20881 infusion and after renal artery constriction (2) was less than that observed with [Sar^1, Ala^8] angiotensin II and with SQ 14225; not until the last day of SQ 20881 infusion was arterial pressure increased but an abrupt further increase in pressure occurred on the day following discontinuation of the drug and this was sustained. This slower and smaller response to renal artery stenosis during SQ 20881 and SQ 14225 compared with [Sar^1, Ala^8] angiotensin II might possibly be related to an increase in the plasma bradykinin level and the associated depressor activity with the SQ compounds. A significant fall in arterial pressure of 9 mm Hg occurred after 24 hr of SQ 14225 administration before the renal artery was constricted. Also, dose response curves to bolus injections of bradykinin revealed a much greater and more prolonged fall in blood pressure during SQ 14225 administration than occurred during the control observations. The smaller response to renal artery stenosis during SQ 20881 compared with SQ 14225 probably reflects more complete blockade of converting enzyme during the continuous infusion of SQ 20881; in the present dogs receiving SQ 14225, arterial pressure was measured before the 8:30 AM dose and this was 10 hr after the previous 10:30 PM dose of the drug. Consequently, a higher drug level might have been present during the SQ 20881 than SQ 14225 at the time of the arterial pressure measurements and resulted in a lower level of arterial pressure.

The responses in PRA were very similar with SQ 20881 and SQ 14225. No increase in plasma aldosterone concentration occurred during SQ 14225 administration and renal artery stenosis, a finding which demonstrates a marked degree of angiotensin blockade. It should be emphasized that SQ 14225 is a competitive antagonist of the converting enzyme so that blockade was never fully complete. Also, from data on the reduction or abolition of the pressor responses to bolus injections of angiotensin I, it is not possible to state the completeness of angiotensin blockade. For example, the degree of blockade necessary to block completely a pressor response to 2 μg of angiotensin I might be more than adequate to achieve 95–98% blockade of the action of the endogenous angiotensin II.

Thus, the use of SQ 14225 to achieve angiotensin blockade provides strong support for the concept that the renin–angiotensin system need never be activated for chronic one-kidney renovascular hypertension to develop and to be sustained. Furthermore, the present study minimizes the importance of the renin–angiotensin system in the pathogenesis of one-kidney hypertension. These findings point out the need to

search for another renal pressor mechanism or a neurogenic mechanism during the development of renovascular hypertension. A promising approach is the recognition of a new hypertensive substance called renopressin which has been described by Skeggs and associates (9).

Summary. Angiotensin blockade was produced one day before and for 7 days after renal artery stenosis in three unilaterally nephrectomized dogs by administration of SQ 14225. The completeness of angiotensin blockade was demonstrated by reduced or absent pressor responses to exogenous angiotensin I and by a normal plasma aldosterone level after renal artery stenosis. A depressor response (9 mm Hg) during the day before renal artery constriction and markedly exaggerated depressor responses to exogenous bolus injections of bradykinin after several days of SQ 14225 administration might reflect an increased plasma level of bradykinin but additional studies are needed on this idea. The arterial pressure response to renal artery constriction was markedly diminished during SQ 14225 and arterial pressure was not significantly elevated until the fifth day. PRA was elevated throughout the period of SQ 14225 administration and renal artery constriction. These findings confirm the earlier observations that the renin-angiotensin system need never be activated for the development and maintenance of chronic one-kidney renovascular hypertension.

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1. Watkins, B. E., Davis, J. O., Freeman, R. H., and Stephens, G. A., Fed. proc. **36,** 532 (1977) (Abstract).
2. Watkins, B. E., Davis, J. O., Freeman, R. H., Stephens, G. A., and DeForrest, J. M. The Physiologist **20,** 99 (Abstract) (1977).
3. Ondetti, M. A., Rubin, B., and Cushman, D. W., Science **196,** 441 (1977).
4. McCaa, R. E., Int. Res. Commun. System **5,** 207 (1977).
5. Sealey, J. E., Laragh, J. H., Gerten-Banes, J., and Aceto, R. M., Hypertension Manual, p. 621 Dun-Donnelley Publishing Corporation, New York (1974).
6. Bühler, F. R., Sealey, J. E., and Laragh, J. H., Hypertension Manual, p. 655 Dun-Donnelley Publishing Corporation, New York (1974).
7. Watkins, B. E., Davis, J. O., Freeman, R. H. and Stephens, G. A., Proc. Soc. Exp. Biol. Med. (In press).
8. Davis, J. O., Circulation Res. **40,** 439 (1977).
9. Skeggs, L. T., Kahn, J. R., Levine, M., Dorer, F. E. and Lentz, K. E., Circulation Res. **40,** 143 (1977).

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