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Inhibition of Angiotensin-Converting Enzyme by SQ 14,225 in Anesthetized Dogs: Hemodynamic and Renal Vascular Effects (40004)

VISHNUBHAKTA S. MURTHY,[1] THOMAS L. WALDRON, AND MORTON E. GOLDBERG

Department of Pharmacology, The Squibb Institute for Medical Research, P.O. Box 4000, Princeton, New Jersey0 08540

Inhibition of vasomotor and endocrine effects of the renin-angiotensin system by suppressing renin release, preventing conversion of angiotensin I (AI) to angiotensin II (AII), or blocking the receptor sites for AII with specific antagonists has received considerable attention for the diagnostic and therapeutic management of hypertensive diseases. Competitive inhibition of angiotensin-converting enzyme (ACE) by a nonapeptide, SQ 20,881, has offered a novel approach to the treatment of hypertension (1-3). However, the therapeutic potential of SQ 20,881 is limited by its lack of oral activity. Recently Ondetti and coworkers (4) have reported the development of SQ 14,225 (D-3-mercapto-2-methylpropanoyl-L-proline), an orally active competitive inhibitor of ACE.

In this investigation, the hemodynamic and renal vascular effects of inhibition of ACE with SQ 14,225 were evaluated in anesthetized dogs.

Methods. Mongrel dogs weighing 10 to 20 kg were anesthetized with sodium pentobarbital (30 mg/kg iv). After endotracheal intubation, artificial respiration was instituted using a Harvard respirator. The right femoral vein was cannulated for intravenous injections, and the right femoral artery was cannulated for the recording of arterial pressure. The heart rate was recorded by triggering a cardiotachometer with arterial pressure pulse. A catheter-tip pressure transducer (Mikro tip, Millar Instruments, Inc., Houston, Texas) was introduced into the left ventricle through the left common carotid artery, and the left ventricular pressure was recorded. The rate of rise of left ventricular pressure (dP/dt) was obtained by electronic differentiation of the left ventricular pressure pulse. The left kidney was exposed using a flank incision and a precalibrated electromagnetic flow probe of suitable size was positioned on the left renal artery. The flow signal was recorded using a Biotronex flowmeter. The ureters were cannulated for timed collection of urine. All signals were recorded on an eight-channel Beckman Dynograph Recorder.

The dogs were divided into four groups. In the first group, consisting of five dogs, effects of increasing doses of SQ 14,225 on the pressor and renal vasoconstrictor effects of AI (310 ng/kg) and AII (100 ng/kg) were investigated. After obtaining control responses to AI and AII, SQ 14,225 was administered iv in half-log dose increments (10 to 310 μg/kg) at 30-min intervals and after each dose the responses to AI and AII were studied. In the second group of four dogs, the duration of ACE inhibition after 310 μg/kg iv of SQ 14,225 was investigated. Responses to AI were studied before and at 10-min intervals after an iv dose of 310 μg/kg of SQ 14,225 for 120 min. In the third group of eight dogs, the cardiovascular and renal effects of a single iv dose of 310 μg/kg of SQ 14,225 were investigated. In the last group of four dogs, cardiovascular and renal effects of 5 ml of saline (solvent) were studied. Also the effects of the solvent on the responses to AI and AII were investigated in this group. AI (Asp^1-Ile^5 angiotensin I) and AII (Asp^1-Ile^5 angiotensin II) were injected iv as a bolus while SQ 14,225 was dissolved in 5 ml of saline and infused over a 5-min period. AI and AII were purchased from Schwarz/Mann (Orangeburg, N.Y.). At the end of the experiment the kidney was removed and weighed.

The results are presented as the mean ± SE in the text, table, and figures. Differ-

[1] To whom correspondence should be addressed.

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ences were considered significant at a level of $P < 0.05$, using Student's t test for paired data.

Results. The resting renal blood flow was 4.1 ± 0.3 ml/min/g of kidney. Intravenous injection of AI and AII caused comparable increases in mean arterial pressure, decreases in renal blood flow, and increases in renal vascular resistance (Fig. 1). After administration of SQ 14,225, a dose-dependent inhibition of the pressor as well as renal vasoconstrictor effects of AI was seen, while saline had no effect (Fig. 2). The renal vasoconstrictor effects were more responsive ($ID_{50} < 10$ μg/kg) to inhibition than the pressor effects ($ID_{50} = 37$ μg/kg). Maximal inhibition of the pressor and the vasoconstrictor effects were seen after 100 and 310 μg/kg of SQ 14,225. The pressor responses to AII were not effected by SQ 14,225 while the vasoconstrictor effects were enhanced (Fig. 2). There was marked variation in the magnitude of potentiation of the vasoconstrictor effects of AII in the individual animals. The animals treated with saline showed no changes in the pressor and vasoconstrictor effects of AII.

Figure 3 illustrates the duration of inhibition of AI-induced pressor and renal vasoconstrictor responses after 310 μg/kg iv of SQ 14,225. Within 10 min of administration of SQ 14,225 the pressor response to AI was inhibited 78%, while 94% inhibition of the renal vasoconstrictor effect was observed. The responses to AI recovered gradually, reaching 50% of the initial response within 60–70 min.

The hemodynamic and renal vascular effects of a single dose of SQ 14,225 (310 μg/kg iv) are presented in Table I. The ACE inhibitor caused a moderate decrease in arterial pressure within 10 min, and complete recovery was seen within 40 min. At the peak of hypotensive effect, a small increase in the heart rate was seen. The left ventricular dP/dt max remained unchanged. After SQ 14,225, renal blood flow in-

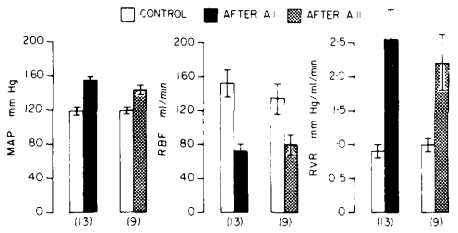

FIG. 1. Effects of angiotensin I (AI, 310 ng/kg, iv) and angiotensin II (AII, 100 ng/kg iv) on mean arterial pressure (MAP), renal blood flow (RBF), and renal vascular resistance (RVR) in anesthetized dogs. Numbers in parentheses equal number of animals.

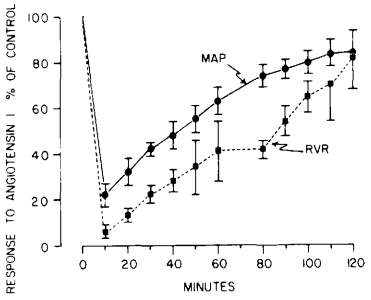

FIG. 3. Temporal changes in the magnitude of angiotensin I (AI, 310 ng/kg iv) induced alterations in mean arterial pressure (MAP) and renal vascular resistance (RVR) after intravenous administration of SQ 14,225 (310 μg/kg) in anesthetized dogs.

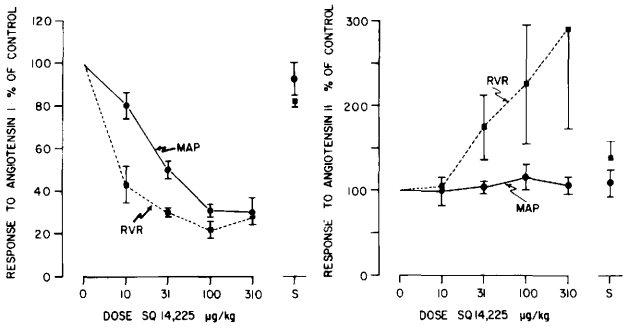

FIG. 2. Effects of intravenously administered SQ 14,225 and saline (s) on the pressor and renal vasoconstrictor effects of angiotensin I (AI, 310 ng/kg iv) and angiotensin II (AII, 100 ng/kg iv) in anesthetized dogs. MAP, Mean arterial pressure; RVR, renal vascular resistance.

TABLE I. HEMODYNAMIC AND RENAL VASCULAR EFFECTS OF SQ 14,225.[a]

	SQ 14,225 (n = 8)[b]		Saline (n = 4)[b]	
	Control	10 min[c]	Control	10 min[c]
Systolic BP (mm Hg)	152 ± 5	139 ± 5[d]	146 ± 7	150 ± 7
Diastolic BP (mm Hg)	114 ± 4	96 ± 5[d]	110 ± 3	113 ± 3
Heart rate (beats/min)	145 ± 6	150 ± 7[d]	162 ± 13	157 ± 14
Left ventricular dP/dt max (mm Hg/sec)	2672 ± 191	2757 ± 215	2811 ± 299	2815 ± 340
Renal blood flow (ml/min)	185 ± 11	205 ± 14[d]	115 ± 16	118 ± 17
Renal vascular resistance (mm Hg/ml/min)	0.70 ± 0.03	0.55 ± 0.04[d]	1.13 ± 0.19	1.14 ± 0.19
Urine output (ml/10 min)	2.26 ± 0.33	2.17 ± 0.38	1.62 ± 0.44	1.54 ± 0.38

[a] SQ 14,225 dose: 310.0 μg/kg iv.
[b] n = Number of dogs used in the test.
[c] Ten minutes after infusion of SQ 14,225 or saline.
[d] $P < 0.05$.

creased by 11% above control value and did not return to control level up to 60 min. The renal vascular resistance decreased. Urine output remained unchanged. Intravenous administration of saline had no effects on any of the parameters studied.

Discussion. The release of renin from the kidneys regulates the vasomotor and endocrine influences of the renin-angiotensin-aldosterone axis. The ACE, a peptidyldipeptide hydrolase, converts the biologically inactive AI to AII, the most potent endogenous vasoconstrictor known (5). SQ 14,225 has been found to be a potent competitive inhibitor ($K_i = 1.7 \times 10^{-9}$ M) of the rabbit lung ACE (4, 6). In isolated guinea pig ileum, intact rat (7), and anesthetized cat (8), it inhibited the effects of AI but not those induced by AII. Our results are in agreement with those of the above investigators. In addition, we found enhancement of the renal vasoconstrictor effects of AII after SQ 14,225. Since inhibition of ACE would result in loss of endogenous AII, enhancement of the effects of AII could be due to expansion of free receptor population for exogenous AII as has been suggested by Thurston and Laragh (9).

In our experiments with anesthetized dogs, SQ 14,225 decreased arterial pressure and caused renal vasodilation. In conscious dogs inhibition of ACE with SQ 20,881 has been found to have no effect on renal blood flow (10, 11). However, in anesthetized dogs, renal vasodilation was observed by various investigators (10, 12–14). It appears that renal vascular effects of ACE inhibitors as well as AII receptor antagonists depend upon the level of activity of the renin-angiotensin system. In dogs, barbiturate anesthesia activates the renin-angiotensin system (10, 15, 16) and reduces renal blood flow (10). Furthermore, the renin-angiotensin system is activated in dogs subjected to negative sodium balance. In sodium-depleted dogs either ACE inhibition with SQ 20,881 or AII receptor blockade with saralasin (1-Sar, 8-Ala angiotensin II) causes renal vasodilation (10, 11, 17). It is therefore proposed that in dogs under barbiturate anesthesia endogenously generated AII contributes significantly to resting renal vascular resistance, and inhibition of ACE with SQ 14,225 removes this vasoconstrictor component and causes vasodilation. However, DiSalvo and co-workers did not observe any change in renal blood flow after ACE inhibition with the pentapeptide inhibitor SQ 20,475 (BPP 5a) in anesthetized dogs (18). The reasons for this descrepancy are not understood presently. The dogs which received saline had lower resting renal blood flow and urine output compared to the group receiving the drug (Table I). Since in both treatments the dogs served as their own controls, it is unlikely that this difference could have influenced the results and the conclusions of this study. The decrease in arterial pressure seen in our experiments after SQ 14,225 could also be due to loss of endogenous AII and the associated transient tachycardia, a reflex-mediated response.

Rubin and co-workers have shown that SQ 14,225 enhances the hypotensive effects of bradykinin (7), since ACE plays an important role in the inactivation of bradykinin (19). It is therefore possible that endogenously generated bradykinin may also contribute to the renal vasodilation seen after SQ 14,225. Although Miller and co-workers (20) failed to demonstrate any increase in circulating levels of bradykinin after inhibition of ACE with SQ 20,881 in dogs, the role of kinins of renal origin cannot be excluded (14).

Contrary to our observations with SQ 14,225, urine output was found to increase after ACE inhibition with SQ 20,881 (12, 14). This disparity could be due to the state of water balance of the animals, since in both the above studies a continuous saline infusion was maintained.

Summary. The hemodynamic and renal vascular effects of SQ 14,225, a new angiotensin-converting enzyme inhibitor, were studied in mongrel dogs under pentobarbital anesthesia. A dose-related inhibition of the pressor and renal vasoconstrictor effects of AI, but not of AII, were seen after SQ 14,225. After administration of SQ 14,225 (310 μg/kg iv) a moderate decrease in arterial pressure and increase in renal blood flow were observed. It is proposed that the hypotension and renal vasodilation seen after SQ 14,225 could be due to loss of endogenous AII; however, an enhancement of endogenously produced bradykinin cannot be excluded.

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