EFFECTS OF ANGIOTENSIN I CONVERTING ENZYME INHIBITOR (SQ 14,225) ON THE RESPONSES OF BLOOD PRESSURE AND STEROID HORMONE TO ANGIOTENSIN II AND ACTH INFUSION IN HYPERTENSIVE SUBJECTS

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The effects of the converting enzyme inhibitor, SQ 14,225, on the renin-angiotensin system, adrenal function and blood pressure were investigated in 14 hypertensive patients, i.e., 10 with essential hypertension (EH) and 4 with renovascular hypertension (RVH). The mean blood pressure (MBP) and plasma aldosterone showed significant decreases in the EH with normal renin (NR) group and in the RVH group but no significant changes in the EH with low renin (LR) group. Plasma renin activity (PRA) increased significantly in the EH with NR group and in the RVH group but showed no significant change in the EH with LR group. Significant correlations were found between the fall in MBP after SQ 14,225 treatment and the pretreatment levels of PRA or plasma aldosterone. In an ACTH infusion study, the response of plasma aldosterone to ACTH revealed significant decreases after SQ 14,225 administration. In an angiotensin II (A II) infusion study, the response of plasma aldosterone was unchanged after SQ 14,225 administration. However, the pressor responses to A II infusion with SQ 14,225 were significantly higher than those without SQ 14,225.

From these findings, it is concluded that the antihypertensive mechanism of SQ 14,225 may be due mainly to the decrease in levels of endogenous A II and that the reduction in plasma aldosterone after SQ 14,225 administration may be due to reduction of endogenous A II levels by converting enzyme inhibition.

SQ 14,225 (D-3-mercaptop-2-methylpropanoyl-L-proline), a converting enzyme inhibitor, has provided a new approach to assessing the role of the renin-angiotensin system in the pathogenesis of hypertension. The antihypertensive efficacy of SQ 14,225 has suggested a wide therapeutic value in treating sustained hypertensive patients in hypertensive emergencies and in hemodialysis-resistant hypertension. Among the diagnostic features, attempts have been made to establish a useful screening procedure for renovascular hypertension (RVH). Many studies have confirmed the clinical usefulness of SQ 14,225, although the antihypertensive mechanism of this agent remains in debate. Recently, several reports indicated that major action of SQ 14,225 involves inhibition of conversion from angiotensin I (A I) to angiotensin II (A II). However, there have been few reports concerning...
ing changes of adrenal function during treatment with SQ 14,225. The aim of the present study was to investigate the effects of SQ 14,225 on adrenal function in order to clarify the role of aldosterone in the antihypertensive mechanism of SQ 14,225 and the influences of SQ 14,225 on the responses of plasma aldosterone to A II and ACTH infusion were studied.

MATERIALS AND METHODS

Subjects
Fourteen hypertensive in-patients, 10 with essential hypertension (EH) and 4 with RVH, including 11 men and 3 women ranging in age from 18 to 61 years (mean = 40.1 ± 2.6 (SE)) were investigated. Their blood pressure (BP) was more than 160 mmHg in systole and/or more than 95 mmHg in diastole in the sitting position. Diagnoses were based on complete physical examinations, serum electrolytes, plasma cortisol and aldosterone, urinary catecholamines and renal angiography (one low renin EH and 4 RVH). The patients with EH were divided into low, normal and high renin groups according to the responses of their plasma renin activity (PRA) after a 2-hour ambulation following intravenous administration of 0.7 mg/kg of furosemide as reported previously. Based on this criterion, 4 of them were judged as low renin EH (LR) and 6 as normal renin EH (NR). Four patients with RVH formed the high renin group.

All antihypertensive medications were discontinued at least 3 weeks before admission except untreated patients. All subjects were studied on a diet containing daily 7–8 g NaCl. All test procedures were performed after a control period of one week.

Test Procedures
The effects of SQ 14,225 on BP, heart rate (HR) and endocrine changes were investigated on the 7th day after administration of SQ 14,225 (75–100 mg/day) in the 14 hypertensive patients. Changes in BP, HR, PRA, and plasma aldosterone and cortisol were evaluated by comparing the average of each value at 6:00, 16:00 and 24:00 (circadian rhythm) on the 7th day after administration of SQ 14,225 with that of pretreatment values.

ACTH Infusion Test
This test was performed before and after administration of SQ 14,225 (75–100 mg/day) in 4 patients with LR, 5 patients with NR and 4 patients with RVH. Immediately after baseline sample had been drawn at 8:00 after a 2-hour recumbency, a 4-hour infusion of 25 I.U. of ACTH (Acthar, Armour Pharmaceutical Co.) in 500 ml of 5% xylitol was begun and 3 other samples were collected one, 2 and 4 hours later. The subjects remained recumbent throughout the study. After 10 days of administration of SQ 14,225, ACTH infusion tests were again performed as described above. SQ 14,225 was given orally at 7:00 before ACTH infusion.

A II Infusion Test
This test was performed before and after administration of SQ 14,225 (75–100 mg/day) in 3 patients with LR, 6 patients with NR and 2 patients with RVH. Immediately after the baseline sample had been drawn at 13:00 after a 2-hour recumbency, A II (Hypertensin, Ciba) dissolved in 500 ml of 5% xylitol was infused at a rate of 8 ng/kg/min for 45 min and plasma samples were collected at 15, 30 and 45 min. BP was monitored at 2-min intervals with an automatic BP recorder (Nihon Cohlin BP-203X) throughout the infusion. Also, in order to assess changes of hepatic blood flow during the infusion, indocyanine green (ICG) infusion was carried out before and after the infusion. The subjects remained recumbent throughout the study. After 7 days of administration of SQ 14,225, A II infusion tests were again performed as described above. SQ 14,225 was given orally at 12:00 before the A II infusion.

The disappearance rate constant of ICG (K) was assessed by the method of Messerli et al. PRA, and plasma aldosterone and cortisol were measured using the RIA-kit of CEA-IRESORIN. Plasma deoxycorticosterone (DOC) and corticosterone were measured by RIA after purification by the method of Honda.

Statistical comparisons were made using Student’s t test for paired data.

RESULTS
The changes in MBP, HR, PRA, and plasma levels of aldosterone and cortisol after administration of SQ 14,225 (75–100 mg/day, for 7 days) are summarized in Table I. MBP and plasma aldosterone decreased significantly in the NR and RVH groups but no significant change was found in the LR group. The HR and plasma cortisol did not change appreciably after administration of SQ 14,225 in any group. PRA increased sig-
**TABLE I** EFFECTS OF SQ 14,225 ADMINISTRATION ON MBP, HR, PRA, AND PLASMA LEVELS OF ALDOSTERONE AND CORTISOL IN HYPERTENSIVE PATIENTS

<table>
<thead>
<tr>
<th></th>
<th>Low PRA (n = 4)</th>
<th>Normal PRA (n = 6)</th>
<th>RVH (n = 4)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>before</td>
<td>after</td>
<td>before</td>
</tr>
<tr>
<td>MBP (mmHg)</td>
<td>111.0 ± 3.1</td>
<td>116.9 ± 3.2</td>
<td>121.8 ± 5.4</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>70.7 ± 3.9</td>
<td>72.9 ± 3.9</td>
<td>71.8 ± 7.6</td>
</tr>
<tr>
<td>PRA (ng/ml/h)</td>
<td>0.5 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>2.2 ± 0.4</td>
</tr>
<tr>
<td>Aldosterone (ng/dl)</td>
<td>7.0 ± 1.7</td>
<td>5.9 ± 0.8</td>
<td>12.5 ± 2.1</td>
</tr>
<tr>
<td>Cortisol (μg/dl)</td>
<td>7.9 ± 2.3</td>
<td>6.9 ± 1.9</td>
<td>9.9 ± 2.2</td>
</tr>
</tbody>
</table>

Values are given as mean ± SE. n = number of determinations.
•: p < 0.05, •••: p < 0.01, for comparisons of values between before and after SQ 14,225 administration.

**TABLE II** EFFECTS OF ACTH INFUSION ON ADRENAL STEROID HORMONES BEFORE AND AFTER SQ 14,225 ADMINISTRATION IN HYPERTENSIVE PATIENTS

<table>
<thead>
<tr>
<th></th>
<th>Time (hour)</th>
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<tbody>
<tr>
<td></td>
<td>before</td>
</tr>
<tr>
<td>SQ 14,225</td>
<td></td>
</tr>
<tr>
<td>DOC (ng/dl) (+)</td>
<td>20.2 ± 2.6</td>
</tr>
<tr>
<td>Corticosterone (ng/ml) (+)</td>
<td>3.6 ± 0.8</td>
</tr>
<tr>
<td>Aldosterone (ng/dl) (+)</td>
<td>16.6 ± 2.4</td>
</tr>
<tr>
<td>Cortisol (μg/dl) (+)</td>
<td>13.0 ± 1.9</td>
</tr>
</tbody>
</table>

Values are given as mean ± SE. ACTH (25 IU) was infused for 4 hours.
**: p < 0.01, as compared to basal levels.

significantly in the NR and RVH groups but no significant change was found in the LR group. Significant correlations were observed between the fall in MBP and the pretreatment PRA values (\( y = -2.33x - 7.47, r = 0.82, p < 0.02 \) in the NR group, and \( y = -6.27x + 6.89, r = 0.88, p < 0.01 \) in the RVH group), and between the fall in MBP and the pretreatment levels of plasma aldosterone (\( y = -0.65x - 4.77, r = 0.79, p < 0.05 \) in the NR group and \( y = -2.54x + 6.73, r = 0.79, p < 0.05 \) in the RVH group). There were, however, no correlations between the fall in MBP and increase in PRA or decrease in plasma aldosterone, respectively.

**ACTH Infusion Test**

The changes in plasma levels of DOC, corticosterone, aldosterone and cortisol after ACTH infusion with and without SQ 14,225 administration, are summarized in Table II. The mean levels of plasma DOC, corticosterone, aldosterone and cortisol after one, 2 and 4 hours of ACTH infusion with and without SQ 14,225 administration were significantly higher than their mean baseline levels, respectively. The baseline levels of plasma aldosterone decreased significantly after SQ 14,225 administration and the incremental responses in plasma aldosterone to ACTH infusion with SQ 14,225 were significantly lower.
TABLE III  EFFECTS OF ANGIOTENSIN II ON MBP, PRA AND PLASMA ALDOSTERONE BEFORE AND AFTER SQ 14,225 ADMINISTRATION IN HYPERTENSIVE PATIENTS

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>before</th>
<th>15</th>
<th>30</th>
<th>45</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBP (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(-)</td>
<td>110.8 ± 4.7</td>
<td>132.3 ± 2.9</td>
<td>128.4 ± 3.2</td>
<td>130.0 ± 5.3</td>
</tr>
<tr>
<td>(+)</td>
<td>97.4 ± 4.2</td>
<td>126.9 ± 4.4</td>
<td>124.6 ± 3.5</td>
<td>126.3 ± 2.6</td>
</tr>
<tr>
<td>PRA (ng/ml/h)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(-)</td>
<td>3.6 ± 1.8</td>
<td>2.9 ± 1.5</td>
<td>2.1 ± 1.0</td>
<td>2.2 ± 1.1</td>
</tr>
<tr>
<td>(+)</td>
<td>7.6 ± 3.0</td>
<td>6.8 ± 2.9</td>
<td>5.2 ± 2.2</td>
<td>4.5 ± 2.0</td>
</tr>
<tr>
<td>Aldosterone (ng/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(-)</td>
<td>10.2 ± 1.9</td>
<td>15.1 ± 3.4</td>
<td>16.7 ± 3.4</td>
<td>14.0 ± 2.7</td>
</tr>
<tr>
<td>(+)</td>
<td>6.1 ± 1.3</td>
<td>11.8 ± 2.4</td>
<td>12.3 ± 2.8</td>
<td>14.0 ± 2.8</td>
</tr>
</tbody>
</table>

Values are given as mean ± SE. Angiotensin II (8 ng/kg/min) was infused for 45 min.

* : p < 0.05, ** : p < 0.02, *** : p < 0.01, as compared to basal levels.

Fig.1. Responses of MBP to angiotensin II infusion before and after SQ 14,225 administration in hypertensive patients. Angiotensin II (8 ng/kg/min) was infused for 45 min.

than those of plasma aldosterone to ACTH infusion without SQ 14,225 administration. The maximal response of plasma aldosterone to ACTH appeared one hour later without SQ 14,225, while appeared 4 hours later with SQ 14,225.

A II Infusion Test

The baseline levels of MBP showed significant decreases (p < 0.05) after SQ 14,225 administration (Table III). MBP after 15, 30 and 45 min of infusion with or without SQ 14,225 administration were significantly higher than each of the mean baseline levels, respectively. Figure 1 shows the changes in BP in terms of the increase in MBP from the baseline levels with and without SQ 14,225 administration. The percent increases of MBP at 15 min from the baseline levels with and without SQ 14,225 administration were 30.4% and 19.4% respectively, and the pressor...
responses to A II infusion with SQ 14,225 were significantly higher than those without SQ 14,225. The mean levels of PRA after 30 and 45 min of A II infusion without SQ 14,225 were significantly lower than the mean baseline level. After SQ 14,225 administration, the baseline levels of PRA showed significant increases. The mean levels of PRA after 15, 30 and 45 min of infusion with SQ 14,225 were significantly lower than the mean baseline level. The mean levels of plasma aldosterone after 15, 30 and 45 min of infusion with and without SQ 14,225 were significantly higher than the mean baseline level. The baseline levels of plasma aldosterone were significantly decreased after SQ 14,225 administration. The percent increase in plasma aldosterone after A II infusion did not change in spite of administration of SQ 14,225.

The mean values of K before and after A II infusion without SQ 14,225 were 0.197 ± 0.008 (min⁻¹) and 0.175 ± 0.010 respectively, and the values of K after the infusion were significantly decreased (p < 0.02). The mean values of K before and after A II infusion with SQ 14,225 were 0.211 ± 0.016 and 0.178 ± 0.012 respectively, and the values of K after the infusion were significantly decreased (p < 0.05). The percent decreases in K after A II infusion with or without SQ 14,225 administration were 15.7% and 11.7% respectively.

**DISCUSSION**

In the present study, the magnitude of the blood pressure reduction after SQ 14,225 administration was found to correlate with the pretreatment PRA levels (Table I). This finding may support the hypothesis that a major mechanism of the antihypertensive action of SQ 14,225 involves the blocking of A II formation. On the other hand, several studies have indicated that SQ 14,225 lowered blood pressure not only in normal and high renin hypertension but also in low renin hypertension, and the blood pressure reduction in low renin hypertension might be due in part to bradykinin accumulation or to the release of endogenous vasodilating prostaglandins. If these vasodilator agents (i.e., bradykinin and prostaglandins) play a major role in the antihypertensive mechanism, it could be speculated that the responses of blood pressure to exogenous A II may be attenuated during SQ 14,225 treatment on account of having a resistance to the vasoconstrictor action of A II. To clarify whether or not the vasodilator action of SQ 14,225 was caused by blocking of A II formation or by bradykinin accumulation, the blood pressure response to exogenous A II before and after SQ 14,225 administration was investigated. The pressor response to A II infusion showed a significant increase after SQ 14,225 administration (Table III and Fig. 1). It is thought that SQ 14,225 administration enhanced the pressor responsiveness to A II. Similar results have been obtained in animal experiments, and it was speculated that the changes in pressor sensitivity were mediated by the vacant receptor of A II not occupied by endogenous A II. Messerli et al. reported that the hepatic clearance of ICG depended entirely on the hepatic blood flow. In the study of ICG test, we observed that the values of ICG disappearance rate constant were significantly decreased after A II infusion, and the reduction in mean value of ICG disappearance rate constant after SQ 14,225 administration was greater than that in the control period. These results suggest that the metabolic clearance rate of A II as well as hepatic blood flow might further decrease during A II infusion after SQ 14,225 administration. Thus, we could further confirm that the vasoconstrictor effect of A II was enhanced after SQ 14,225 administration (Table III and Fig. 1). Jaeger et al. reported that teprotide (SQ 20,881) could not involve a mechanism of systemic vasodilation due to accumulation of endogenous bradykinin, since teprotide failed to cause a reduction in the increased blood pressure due to infused A II in the experiment of combined infusion of teprotide and A II in rats. Accordingly, the enhanced pressor responses to A II infusion with SQ 14,225 may provide additional indirect evidence against a potential depressor effect due to bradykinin accumulation as discussed by Jaeger et al.

Recently, Murthy et al. reported that inhibition of converting enzyme may cause an increase in the release of prostaglandins (PGs) in addition to enhancing the effect of bradykinin itself and PGs are thought to be involved in mediating the hypotensive response of SQ 14,225. Terrano et al. reported that bradykinin selectively increased the synthesis of PGE-like substance in the arteries. Moreover, their group revealed that PGE₂ attenuated the vascular actions of pressor hormones (A II and norepinephrine). If such release of PG could be caused by inhibition of converting enzyme, the enhancement of the vasoconstrictor effect of A II after SQ 14,225 should be diminished. In the present study, SQ
14,225 administration enhanced the pressor responsiveness to AII (Table III and Fig. I). Therefore, we speculate that the depressor effect of SQ 14,225 may be not due to the accumulation of bradykinin and/or the release of PGs.

Concerning the effect of SQ 14,225 on the adrenal gland, only the concentrations of plasma aldosterone decreased significantly after SQ 14,225 administration. To assess whether or not the reduction in plasma aldosterone might be due to the direct effect of SQ 14,225 on adrenal steroid biosynthesis, ACTH infusion tests were performed. In the case without SQ 14,225 administration, the levels of plasma aldosterone increased rapidly within one hour after ACTH infusion and reached a plateau thereafter. On the other hand, the levels of plasma aldosterone in response to ACTH after SQ 14,225 administration were increased significantly one hour after the infusion but the maximal response to ACTH was prolonged for 4 hours after the infusion. In addition, the response of plasma corticosterone to ACTH 4 hours after SQ 14,225 administration was remarkably increased when compared to that without SQ 14,225. Based on these findings, it can be suggested that SQ 14,225 may affect the late step (corticosterone to aldosterone) in adrenal steroid biosynthesis. In an AII infusion study, the incremental response of plasma aldosterone to AII infusion did not change after SQ 14,225 administration. It is considered therefore that the reduction in plasma aldosterone after SQ 14,225 administration may be due to a direct inhibitory effect of SQ 14,225 on the adrenal gland but to reduction of the endogenous A II level by converting enzyme inhibition.

In conclusion, the antihypertensive mechanism of SQ 14,225 may be due mainly to the decrease in levels of endogenous A II and the reduction in plasma aldosterone after SQ 14,225 administration may be due to reduction of endogenous A II levels by converting enzyme inhibition.

REFERENCES


