The Effect of SQ14,225 on Systolic Blood Pressure and Urinary Excretion of Vasopressin in the Developing Spontaneously Hypertensive Rat

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SUMMARY In order to assess the relationship in the spontaneously hypertensive (SH) rat between the development of hypertension and the increased secretion of vasopressin which we have shown in this model, SH rats were treated with the orally effective converting enzyme inhibitor, SQ14,225 (15 to 30 mg/kg-day in the drinking water), from ages 33 to 61 days. Systolic blood pressure (SBP) in the treated rats increased from a pretreatment level of 112 ± 6 (SE) mm Hg to only 136 ± 2 mm Hg by the fourth week of treatment. In the untreated SH rats SBP rose from 116 ± 5 mm Hg to 174 ± 4 mm Hg in this same period. Within 2 days of initiating treatment with SQ14,225, 24-hour urinary excretion of ADH \( (U_{ADH}) \) fell 46% and remained depressed for the duration of treatment, while in the untreated rats \( U_{ADH} \) tended to increase. Treatment with SQ14,225 gradually increased water intake to a level 64% higher than in the untreated rats at the end of 4 weeks. However, the fall in \( U_{ADH} \) appeared to precede the increase in water intake. The changes in \( U_{ADH} \) and water intake were reflected by an increased urine volume and decreased urine osmolality. All changes were reversed within 9 days after discontinuing treatment with SQ14,225. The decreased release of vasopressin, suggested by the decreased \( U_{ADH} \), could have been a factor in preventing the development of hypertension in the treated rats to the extent that vasopressin acts as a pressor agent in the SH rat and that blood volume was reduced as a result of the increased urine volume. (Hypertension 1: 462–467, 1979)

KEY WORDS • vasopressin • hypertension • captopril • SQ14,225 • SH rat • antidiuretic hormone • blood pressure

In the past several years, evidence has accumulated that vasopressin plays a pressor role in the development and/or maintenance of the elevated blood pressure in DOC-salt hypertension,1,2 two-kidney Goldblatt hypertension,3 and in the malignant phase of hypertension in the stroke-prone spontaneously hypertensive rat on a high salt intake.4 We have recently reported4 that there is an increased secretion of vasopressin in the young spontaneously hypertensive (SH) rat of the Okamoto-Aoki strain. However, because the increase in the plasma vasopressin concentration was relatively small, and since a competitive antagonist of the pressor action of vasopressin had only a small effect on arterial blood pressure in the 10-week-old SH rat, the question of the relationship between the development of hypertension and the increased secretion of vasopressin in the SH rat remained.

In the present experiments, we have explored this issue further by preventing the development of hypertension in the young SH rat by treatment with the orally active converting enzyme inhibitor,5 SQ14,225 (captopril), and determining the effect on the release of vasopressin. The 24-hour urinary excretion of
vasopressin was used as a convenient index of release of vasopressin from the neurohypophysis.

Methods

Sixteen male spontaneously hypertensive rats of the Okamoto-Aoki strain (SH) were obtained from Charles River Breeding Laboratories when they were 27 days old. The rats were housed individually in stainless steel metabolism cages under continuous lighting. Room temperature was held between 22 and 24°C. Purina Laboratory Chow and deionized water were allowed ad libitum.

When the rats were 30 days old, measurements of systolic blood pressure (SBP) and the 24-hour urinary excretion of vasopressin and water were begun. Systolic blood pressure was measured by tail plethysmography in the conscious rat warmed at 30°C for 15 to 20 minutes. Each measurement of SBP followed a 24-hour urine collection period. Control measurements were obtained at ages 30 and 33 days. After the latter measurement, the rats were separated randomly into two groups: eight rats were given SQ14,225 (15 to 30 mg/kg-24 hrs) in the drinking water, and eight rats received deionized water to drink. The solution of SQ14,225 was prepared each day. Body weight and the amount of fluid ingested were measured daily and averaged. From these data, we were able to predict what the rat would weigh and how much it would drink the following day. The amount of SQ14,225 needed to achieve the 15 to 30 mg/kg-day dose was calculated and added to the amount of water we anticipated the rat would drink. Systolic blood pressure, the urinary excretion of vasopressin, sodium, potassium and water, and urine osmolality were measured 48 hours (age 35 days) and 72 hours (age 37 days) after initiation of treatment with SQ14,225. Because of limited urine volume, electrolyte and osmolality measurements were not obtained during ages 30 and 33 days, in order to allow sufficient sample for vasopressin analysis. Beginning at age 40 days and continuing through age 61 days, when treatment with the drug was discontinued, systolic blood pressure and urine excretion were measured weekly. Final measurements were taken at age 70 days, 9 days after discontinuing the drug.

The methods for the collection of urine samples and for the extraction and radioimmunoassay of urinary vasopressin are described elsewhere.

Recovery of vasopressin from the urine of SH animals treated with SQ14,225 and from that of nontreated animals was determined each time vasopressin was extracted from urine. Average recovery for the SH-SQ14,225 animals was 79.5 ± 3.1% (se; n = 7); average recovery for the nontreated animals was 76.4 ± 5.6% (n = 7). Values for urinary vasopressin excretion were not corrected for incomplete recovery. Beginning at age 35 days, an aliquot of each urine sample was saved for the measurements of urinary excretion of sodium and potassium and urine osmolality. Urinary sodium and potassium were measured with an IL343 flame photometer. Urinary osmolality was measured by freeezing point depression with an Osmette A osmometer.

A two-factor analysis of variance for repeated measures was used to determine whether statistically significant differences with time within each group and differences between treated and nontreated groups existed. A Newman-Keuls a posteriori test was used to isolate differences found with time; multiple one-factor analyses of variance for independent measures were used to isolate differences between groups. Means are given ± one standard error in the text, tables, and figures, but the standard errors were not used for the statistical analyses.

Results

By age 61 days, systolic blood pressure (fig. 1A) in the untreated SH rats had increased an average of 73 mm Hg (p < 0.01) over that measured at age 30 days, whereas systolic blood pressure increased only 30 mm Hg in the SQ14,225-treated rats. When treatment with SQ14,225 was discontinued and both groups of animals were tested 9 days later, systolic blood pressure in the treated group had risen to levels seen in the nontreated group. During treatment, systolic blood pressure in the SQ14,225-treated group was always significantly lower (p < 0.05 to p < 0.01) than in the nontreated group.

The 24-hour urinary excretion of vasopressin (fig. 1B) was slightly higher in the SQ14,225-treated than in the nontreated animals during the two collection periods preceding administration of the drug, but this difference was statistically significant (p < 0.05) only at age 30 days. Within 2 days after treatment was begun, vasopressin excretion fell 46% (p < 0.01), and remained at this low level until age 47 days. It fell an additional 65% (p < 0.01) at age 54 days. The urinary excretion of vasopressin in the nontreated SH rats tended to increase with age, but this effect was statistically significant (p < 0.01) only at ages 40, 47, and 70 days. From age 40 through 61 days, vasopressin excretion in the treated rats was two to three times that in the rats receiving SQ14,225 (p < 0.01). Nine days after SQ14,225 was withdrawn, the urinary excretion of vasopressin was virtually identical in both groups.

Fluid intake (fig. 2A) increased (p < 0.01) in both groups with time. Within 4 days after starting SQ14,225, the treated group consumed 14% more fluid (p < 0.01) than the untreated rats. However, fluid intake, while remaining consistently greater in the treated animals, did not increase substantially until the final week of treatment, when the SQ14,225-treated rats drank 64% more water than the nontreated rats. The increase in fluid intake and the decreased excretion of vasopressin were accompanied by an increased urine volume (fig. 2B) and a decreased urine osmolality (fig. 2C). When SQ14,225 was withdrawn, water intake and urine volume and osmolality returned to levels seen in the nontreated animals. The SQ14,225-treated animals also exhibited an increased (p < 0.05) 24-hour excretion of sodium (table 1), again most pronounced at age 61 days when the treated
animals excreted 34% more sodium than the untreated animals. There were no significant differences in potassium excretion between the two groups (table 1).

Body weight (table 1) increased progressively \((p < 0.01)\) in both groups with time. The treated group was slightly heavier \((p < 0.05)\) than the untreated group until age 40 days. After age 40 days there were no significant differences in body weight between the two groups.

Discussion

In the present experiments, we have found that treatment of SH rats with the orally active converting enzyme inhibitor, SQ14,225, beginning at age 33 days, greatly reduces the increase in blood pressure that is otherwise seen in these animals. After 4 weeks of treatment, systolic blood pressure had only risen to levels that we had previously found in normotensive WKY rats of corresponding age. In contrast, treatment of 10- to 14-week-old SH rats with SQ14,225 for 6 months is required to lower arterial blood pressure to normotensive levels. The most obvious and, perhaps, most likely mechanism for this action of SQ14,225 is the blockade of the conversion of angiotensin I to angiotensin II, thereby lowering plasma levels of the latter. Consistent with this interpretation is the recent report by Muñoz-Ramírez et al.
al.\(^4\) that blockade of angiotensin II with [sarcosine-1, threonine-8] angiotensin II, an antagonist with minimal agonistic activity, acutely lowered arterial blood pressure in SH rats anesthetized with inactin. On the other hand, the question of whether plasma renin levels are elevated in the SH rat is highly controversial. However, plasma renin levels may not adequately reflect the role of the renin-angiotensin system in this form of hypertension. One must consider whether the observed plasma renin level is appropriate for the state of sodium balance and of the arterial blood pressure. Furthermore, Hoffman et al.\(^9\) have demonstrated that there is an increased pressor sensitivity to angiotensin II in the SH rat. Alternatively, the possibility that SQ14,225 exerts its action on arterial blood pressure via the renal kallikrein-kinin system must also be considered.

We have previously shown\(^4\) that in the young SH rat the urinary excretion of vasopressin, the plasma vasopressin concentration, and the pituitary content of vasopressin are elevated. In the present experiments, administration of SQ14,225 to young SH rats markedly reduced the urinary excretion of vasopressin throughout the 4-week period of treatment. Whether this was due to an inhibition of release of vasopressin from the neurohypophysis depends on whether changes in the long-term urinary excretion of this hormone reflect changes in vasopressin release. That this may be the case is supported by the facts that the kidney plays a major role in the clearance of vasopressin from the circulation,\(^10\) and that stimuli which affect vasopressin release result in appropriate changes in the urinary excretion of this hormone. Thus, the urinary excretion of vasopressin is decreased following hydration\(^11-15\) and increased following dehydration or infusion of hypertonic saline.\(^11-18\)

There are at least four mechanisms that could be responsible for the inhibition of vasopressin release in rats treated with SQ14,225. The first is a reduction in the plasma angiotensin II concentration. However, the effect of circulating angiotensin II on vasopressin release is controversial. Although many investigators report that angiotensin II stimulates vasopressin secretion,\(^17\) others report that it does not.\(^18\) Accepting that exogenous angiotensin II can stimulate vasopressin release under some conditions, there is some question as to whether endogenously generated angiotensin can reach a plasma level sufficiently high for this effect.\(^19\) A second possible mechanism is that the increased water intake observed in the treated rats could have inhibited vasopressin release via a reduction in plasma osmolality. Although it is likely that this was an important factor in the latter part of the present experiments, when there was a substantial increase in water intake in the drug-treated rats, a reduction in vasopressin excretion was observed before water intake in the SQ14,225-treated animals differed significantly from that in the controls. Furthermore, the magnitude of the effect on water intake was initially small compared to the magnitude of the effect on vasopressin excretion. Third, it is possible that SQ14,225 acted centrally, perhaps at the organum vasculosum of the lamina terminalis or the subfornical organ, to inhibit directly the release of

| Table 1. Twenty-Four Hour Urinary Excretion of Sodium (U_NaV) and Potassium (U_KV) and Body Weight in SH Rats Receiving Either no Treatment or Treatment with SQ14,225 (16 to 30 mg/kg•4 hrs). Treatment with SQ14,225 was Begun Immediately after Measurements at Age 55 Days and Discontinued after Measurements at Age 61 Days. Differences from Age 55 Days are Shown by Daggers Beside the Values; Differences Between Groups are Shown by Asterisks and Daggers Between the Values. |
|---|---|---|---|---|---|---|---|---|---|
| Age (days) | 30 | 33 | 35 | 37 | 40 | 47 | 54 | 61 | 70 |
| U_NaV (mEq/24 hrs • 100 g body weight) | | | | | | | | | |
| Untreated | 1.04 | 0.74 | 0.83 | 0.88 | 0.81 | 0.87 | 0.51 |
| (n = 8) | ±0.09 | ±0.08 | ±0.04 | ±0.07 | ±0.08 | ±0.10 |
| Treated | 1.07 | 0.95 | 0.91 | 0.91 | 0.85 | 1.17 | 0.47 |
| (n = 8) | ±0.05 | ±0.06 | ±0.05 | ±0.05 | ±0.05 |
| U_KV (mEq/24 hrs • 100 g body weight) | | | | | | | | | |
| Untreated | 1.76 | 1.83 | 1.92 | 1.69 | 1.65 | 1.44 | 1.04 |
| (n = 8) | ±0.08 | ±0.09 | ±0.05 | ±0.10 | ±0.10 | ±0.10 | ±0.09 |
| Treated | 1.84 | 2.16 | 2.02 | 1.71 | 1.69 | 1.82 | 1.05 |
| (n = 8) | ±0.10 | ±0.10 | ±0.10 | ±0.14 | ±0.09 | ±0.10 | ±0.09 |
| Body weight (g) | | | | | | | | | |
| Untreated | 45† | 53 | 70† | 79† | 91† | 124† | 159† | 195† | 220† |
| (n = 8) | ±1 | ±1 | ±2 | ±2 | ±3 | ±4 | ±4 | ±4 | ±4 |
| Treated | 55† | 68 | 76† | 85† | 100† | 131† | 155† | 190† | 211† |
| (n = 8) | ±1 | ±1 | ±2 | ±1 | ±2 | ±3 | ±2 | ±4 |

*p < 0.05.
†p < 0.01.
vasopressin from the posterior pituitary. Finally, SQ14,225 could have impaired the ability of the kidney to clear vasopressin, but this does not seem likely. At this time, however, there is insufficient information to decide definitively among these mechanisms. It should be pointed out that it is unlikely that blockade of kininase activity by SQ14,225 was responsible for the reduction in vasopressin excretion since it has been reported that kinins increase vasopressin release.

Of particular interest is the possible relationship between the reduction in the urinary excretion of vasopressin and the prevention of the development of hypertension in the SQ14,225-treated animals. There is evidence that vasopressin plays a role as a pressor agent in DOC-salt hypertension, two-kidney Goldblatt hypertension, and in the stroke-prone SH rat. We have previously provided evidence for an increased release of vasopressin from the neurohypophysis in the young SH rat. However, the increase in the plasma vasopressin concentration in those animals was small, and a competitive antagonist of the pressor action of vasopressin had only a small effect on mean arterial blood pressure in the conscious 10-week-old SH rat. The inhibition of vasopressin release could have contributed to the prevention of the development of the hypertension in another way. A reduction in blood volume secondary to the decreased release of vasopressin and increased water excretion could have been a significant factor in the failure of the treated rats to become hypertensive.

The observation that treatment with SQ14,225 stimulated thirst is paradoxical. It is generally accepted that angiotensin II stimulates thirst, and treatment with SQ14,225 almost certainly lowered the plasma concentration of this hormone. It has been reported previously that acute treatment with another converting enzyme inhibitor, SQ20,881, increased water intake in the intact rat and enhanced thirst in water intake in the intact rat. These investigators suggested that the inhibition that intracerebroventricular administration of SQ20,881 prevented the thirst induced by systemic injection of the drug. With respect to the present experiments, one wonders why SQ14,225, a smaller molecule than angiotensin I, could not have reached the same central site to prevent conversion of angiotensin I to angiotensin II. Alternatively, if vasopressin secretion was reduced initially, due either to a reduction in circulating levels of angiotensin II or to a direct effect of SQ14,225 on the central nervous system, the increased water intake could have been secondary to the increased excretion of water by the kidney.

In summary, treatment of the young SH rat with the orally effective converting enzyme inhibitor, SQ14,225, prevented the development of the hypertension, and there was evidence for a decreased secretion of vasopressin. The decreased release of vasopressin could have contributed to the prevention of the hypertension to the extent that vasopressin functions as a pressor agent in the SH rat, and to the extent that blood volume was reduced due to the increased urinary excretion of water. Determination of the importance of these factors awaits further study.

Acknowledgments

The authors wish to express their gratitude to Ms. Cynthia Allen, Ms. Linda K. Long, and Mrs. Deborah Tarnowski for their valuable technical assistance.

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*Hypertension.* 1979;1:462-467
doi: 10.1161/01.HYP.1.5.462

*Hypertension* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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