Effects of Chronic Infusion of Renin Inhibitor A-64662 in Sodium-Depleted Monkeys

Kenneth M. Verburg, Hollis D. Kleinert, Jill R.C. Kadam, Marcia A. Chekal, Peter F. Mento, and Barry M. Wilkes

The potent and primate-selective renin inhibitor A-64662 (n=8) or vehicle (n=6) was administered intravenously for 7 days to sodium-depleted cynomolgus monkeys to investigate the chronic effects on arterial pressure, sodium excretion, and the renin-angiotensin-aldosterone system. A 0.1-mg/kg i.v. bolus followed by a continuous 0.01-mg/kg/min infusion of A-64662 lowered mean arterial pressure from 89±3 (average of 4 control days) to 75±4 mm Hg (p<0.05) after 1 day of administration. This decrement was associated with marked inhibition of plasma renin activity (PRA) from 57.7±11.1 to 1.3±0.6 ng angiotensin I (Ang I)/ml/hr (p<0.05). Similar hypotensive levels (range 73±4 to 77±4 mm Hg) were observed on days 2-7 of A-64662 infusion and PRA remained suppressed, ranging from 0.6±0.4 to 1.9±1.0 ng Ang I/ml/hr. Plasma angiotensin II (Ang II) levels were reduced (p<0.05) from the control value of 66.7±20.2 to 12.4±3.3 and 26.4±6.5 pg/ml on the second and seventh days, respectively, of A-64662 infusion. In contrast, infusion of vehicle alone had no discernible effect on mean arterial pressure, PRA, or plasma Ang II concentrations. Plasma aldosterone decreased (p<0.05) from control on the second and third days of A-64662 infusion, although differences between the treatment groups were not detected throughout the study. Urinary sodium excretion remained at control levels throughout the infusion of A-64662. Cessation of A-64662 administration resulted in a recovery of mean arterial pressure to preinfusion levels within 1 day. This study indicates that continuous infusion of A-64662 results in a sustained hypotension in sodium-depleted monkeys. This effect appears to be related, at least partially, to inhibition of PRA and lower plasma Ang II levels. (Hypertension 1989;13:262-272)

The renin-angiotensin system is recognized to play an important role in normal cardiovascular regulation by virtue of its actions on blood pressure and fluid volume homeostasis. Although the role of the renin-angiotensin system in the etiology of essential hypertension is unclear, the efficacy of angiotensin converting enzyme inhibitors as antihypertensive agents has been firmly established.1,2 This observation has led to considerable interest in the pursuit of compounds that inhibit renin, the enzyme that catalyzes the rate-limiting step in the formation of angiotensin II (Ang II).

Early success in the development of renin inhibitors centered on truncated substrate analogues that contained a modified amino acid sequence surrounding the N-terminal cleavage site of angiotensinogen.3-4 More recently, several renin substrate analogues that function as transition-state mimics have been synthesized and characterized as potent renin inhibitors with IC50 values in the low nanomolar range.5-9 The inhibitory activity of these compounds is imparted by a variety of structures resembling the configuration of the scissile bond of angiotensinogen after activation for cleavage by renin. Most of the renin inhibitors reported to date have in vivo activity and lower blood pressure in sodium-deficient normotensive animals3,4,6-10 or in renin-dependent forms of experimental hypertension4,11 after acute intravenous dosing. When given continuously, chronic administration of some renin inhibitors has also been found to elicit a prolonged hypotension in sodium-depleted animals.10,12

In recent reports, Luly et al13,14 and Kleinert et al15-17 described the synthesis, biochemical characteristics, and efficacy of various dipeptide renin inhibitors culminating in the development of A-64662.
catheters (PE90) were placed in the inferior vena cava and lower abdominal aorta through the left femoral vein and artery, respectively. The distal end of each catheter was then routed subcutaneously to the midscapular region and exteriorized. Both catheters were filled with a heparin solution (1,000 units/ml). Finally, the monkeys were fitted with nylon jackets (Alice King Chatam Medical Arts, Los Angeles, California) to protect the catheters and then returned to their cages. The animals were permitted a 1-week minimum postoperative recovery period. Patency of the catheters was ensured by flushing with saline and refilling with heparin twice a week.

**Experimental Protocol**

Beginning 2 weeks before the first control measurements, the monkeys were placed on a diet consisting of low sodium chow (0.013 meq Na+/g) and fruit. The monkeys were then maintained on this sodium-restricted diet for the duration of the study. Each monkey was also treated with furosemide (10 mg/kg, p.o.) for a 1-week period beginning 5 days after the sodium-restricted diet was instituted and ending 2 days before the first control measurements. Food intake and urinary sodium excretion of seven monkeys were monitored on a daily basis to determine the magnitude of sodium depletion that was achieved by this regimen.

One day before the first control measurements, the monkeys were transferred to chairs as previously described. The arterial catheter was connected to a Model 7B Grass polygraph (Grass Instruments, Quincy, Massachusetts) via a Model P23 Gould-Statham pressure transducer (Gould Inc., Oxnard, California). The venous catheter was connected to a syringe infusion pump (model 355, Sage Instruments, Cambridge, Massachusetts) for the continuous infusion of 5% dextrose in H2O (D5W, Abbott) at a rate of 0.2 ml/hr. Four consecutive daily control measurements were obtained. Immediately after the last control measurement, A-64662 was infused intravenously into eight randomly assigned monkeys beginning with a 0.1-mg/kg bolus and followed by a continuous infusion of A-64662 at a rate of 0.01 mg/kg/min. The initial effect of A-64662 administration on arterial pressure and heart rate was continuously monitored for a 2-hour period in each monkey. A-64662 was prepared at a concentration of 100 mg/ml in 0.3 M acetic acid. D5W was used for additional dilutions and served as the vehicle in the six monkeys that were used as the control group. A-64662 was continuously infused for 7 days before a 3-day recovery period was observed. Arterial pressure was monitored in all monkeys for a 1-hour period between 8:00 and 11:00 AM each day of the study. The data were recorded on magnetic tape and processed subsequently by a PDP-11 computer to determine the average daily arterial pressure and heart rate for each monkey. After the hemodynamic measurements were determined, a 1.7-ml arterial blood sample was obtained and replaced with an equivalent volume of a replace-

Materials and Methods

Male cynomolgus monkeys (Macaca fascicularis, Hazelton, Reston, Virginia) weighing 3–6 kg were housed under constant lighting and temperature conditions and were maintained on normal monkey plasma renin and 14 nM for human plasma renin at pH 7.4. Furthermore, single intravenous bolus injections of A-64662 were shown to induce a dose-related reduction in blood pressure accompanied by inhibition of plasma renin activity (PRA) in both sodium-depleted and sodium-replete anesthetized monkeys. 17

The present study was designed to investigate further the characteristics of A-64662 by examining the effects of this renin inhibitor when chronically administered as a continuous 7-day intravenous infusion to normotensive, sodium-depleted monkeys. The primary objective of this investigation was to determine whether a chronic blockade of the renin-angiotensin system by A-64662 could be maintained leading to a prolonged hypotension and to establish the temporal relation between changes in arterial pressure, excretory function, and inhibition of the renin-angiotensin system produced by A-64662 administration.

Surgical Preparation

Monkeys deprived of food overnight were sedated with ketamine hydrochloride (Bristol, Syracuse, New York) 10 mg/kg i.m. Catheter implantation was carried out under sodium pentobarbital (Abbott Laboratories, North Chicago, Illinois) anesthesia produced by a bolus dose of 15 mg/kg i.v. and then maintained by a 0.1 mg/kg/min constant infusion. Surgery was performed under aseptic conditions using sterile techniques. Silastic-coated polyethylene catheters (PE90) were placed in the inferior vena cavas and lower abdominal aortas, respectively. The distal end of each catheter was then routed subcutaneously to the midscapular region and exteriorized. Both catheters were filled with a heparin solution (1,000 units/ml). The monkeys were fitted with nylon jackets (Alice King Chatam Medical Arts, Los Angeles, California) to protect the catheters and then returned to their cages. The animals were permitted a 1-week minimum postoperative recovery period. Patency of the catheters was ensured by flushing with saline and refilling with heparin twice a week.

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ment electrolyte solution (Normosol-R, Abbott). On four occasions during the study, an additional 3.0-ml arterial blood sample was withdrawn for the determination of plasma Ang II levels. After daily measurements were obtained, the acute blood pressure response to an intravenous bolus administration of captopril (0.1 mg/kg) or additional A-64662 (2.0 mg/kg) was monitored on the second and seventh days of the infusion, respectively, in five monkeys receiving A-64662 chronically. The animals were fed each day immediately after the blood sample was obtained. Daily food intake was monitored and 24-hour urine specimens were collected each day at approximately 6:00 AM.

Analytical Procedures

A 1.2-ml aliquot of each arterial blood sample was placed in tubes containing 15 mg EDTA. The remaining 0.5 ml of blood was placed in heparinized tubes. Blood samples were centrifuged at 1,500g for 15 minutes at room temperature. The plasma was removed, aliquoted, and stored at -20° C. PRA was determined by a method previously described. Briefly, PRA was measured as the rate of angiotensin I (Ang I) formation at pH 5.7 during a 3-hour incubation at 37° C. Antisera to Ang I was obtained from rabbits immunized intradermally after conjugation of Ang I with keyhole limpet hemocyanin (Calbiochem, San Diego, California). Plasma aldosterone concentrations were quantified by combining high-performance liquid chromatographic (HPLC) separation of Ang I with keyhole limpet hemocyanin (Calbiochem, San Diego, California) in the presence of glutaraldehyde. A 1:400 dilution of this antisera yielded 60-65% binding of [125I]Ang I (NEN Research Products, Boston, Massachusetts) in the absence of unlabeled Ang I. The plasma aldosterone concentration (PAC) was measured from extracted plasma by using a commercially available radioimmunoassay kit (Abbott). Plasma cortisol levels were also quantified by radioimmunoassay (Diagnostic Products, Los Angeles, California). Plasma and urinary electrolytes were determined by flame photometry. Plasma creatinine was determined by a standard spectrophotometric technique.

Blood samples for Ang II determinations were placed in chilled tubes containing EDTA and immediately centrifuged at 4° C. The plasma was then added to tubes containing disopropyl phosphorofluoridate and stored at -20° C. Plasma Ang II concentrations were quantified by combining high-performance liquid chromatographic (HPLC) separation with radioimmunoassay using a recently described technique with minor modifications in the HPLC separation. One-milliliter plasma samples were extracted, and the extracted residue was dissolved in 60 μl mobile phase for HPLC. The separation of angiotensins was achieved by using a 3.9 mm×15 cm Nova-Pak C18 column (Waters, Milford, Massachusetts) connected to a Perkin-Elmer Series 4 pump system (Perkin-Elmer, Norwalk, Connecticut) fitted with a Rheodyne 7125 manual injection valve (Rheodyne, Cotati, California). Elution was achieved with a gradient from 89% buffer A (25 mM sodium phosphate, 5% acetonitrile, pH 7.8) and 11% solution B (95% acetonitrile) to 68% A over 12 minutes as a flow rate of 1.5 ml/min. Fractions (6 seconds) were collected into 12×75 mm glass test tubes and dried. Plasma Ang II was measured on the appropriate fractions by radioimmunoassay.

Plasma concentrations of A-64662 were quantified by HPLC analysis. Each 100-200 μl plasma sample was adjusted to an alkaline pH by adding 250 μl 0.5 mg NaCO3 containing the renin inhibitor A-63925 ([H-β-ala-phe-his-cyclohexyl]ala(OH)-CHOH-Isobutyl.2HOAC) as an internal standard. The sample was extracted with 5 ml ethyl acetate/hexane (7:3) and then back-extracted into 250 μl 0.1% trifluoroacetic acid (TFA). The aqueous phase was washed with 2 ml hexane, and 80 μl was then injected onto an ODS2 3 μm×5 cm column (Regis, Morton Grove, Illinois) connected to a Spectra-Physics 8780 chromatography/autosampler and 8490 variable wavelength detector operating at 205 nm (Spectra-Physics, San Jose, California). The mobile phase was composed of acetonitrile, methanol, and 0.01 M tetramethylammonium perchlorate (35:5:60) in 0.1% TFA. The detection limit of this procedure was 30 ng/ml.

Statistical Analysis

The results are presented as mean±SEM. The data were analyzed by analysis of variance for repeated measurements and Student’s t test. Differences at the 5% level were considered statistically significant.

Results

Seven monkeys were studied to determine the typical degree of sodium depletion and activation of the renin-angiotensin system produced by the low sodium diet and regimen of furosemide treatments. On the normal diet, sodium intake averaged 8.2±0.9 meq/day and sodium excretion was 7.0±1.1 meq/day for the 5-day period that was examined. PRA and the PAC were 3.9±1.2 ng Ang I/ml/hr and 3.3±0.7 ng%, respectively. When the monkeys were placed on the low sodium chow, dietary sodium intake on a daily basis was reduced to an average of 1.0±0.1 meq. Conversion to the low sodium diet in combination with furosemide administration resulted in a net negative cumulative sodium balance of 25.1±1.2 meq and a reactive rise of PRA and PAC to 66.4±12.0 ng Ang I/ml/hr and 76.2±23.7 ng%, respectively.

The continuous intravenous administration of A-64662, beginning with a 0.1-mg/kg bolus and followed by the infusion of 0.01 mg/kg/min, induced a rapid and sustained hypotension. The initial effects of A-64662 on mean arterial pressure (MAP) and heart rate are shown in Figure 1. Control MAP averaging 87±4 mm Hg decreased significantly to 76±4 mm Hg beginning 5 minutes after the infusion of A-64662 was initiated, and this hypotensive response was maintained with little change as MAP averaged 74±4 mm Hg during the 2-hour observation period. Heart rate was unaffected by A-64662.
FIGURE 1. Line graphs showing initial effects of the intravenous infusion of A-64662 on mean arterial pressure (MAP) and heart rate in sodium-depleted monkeys (n=8). Drug administration began with a 0.1 mg/kg bolus and was followed by a 0.01 mg/kg/min infusion. Values are mean±SEM. *p<0.05 versus control.

infusion as the decrement in MAP did not result in reflex tachycardia. Significant reductions in systolic and diastolic pressure occurred with A-64662 infusion although no diminution of pulse pressure was observed (data not shown).

The systemic hemodynamic effects over the course of the 7-day infusion of A-64662 are illustrated in Figure 2. In the drug-treated group (n=8), MAP averaged between 90±4 and 87±3 mm Hg during the 4-day control period preceding A-64662 infusion. These values closely overlapped with the average MAP of the vehicle-infused group, which ranged from 87±3 to 89±3 mm Hg during the same period. MAP decreased significantly to 75±4 mm Hg after 1 day of A-64662 infusion. Thereafter, MAP stabilized in the range of 73±4 to 77±4 mm Hg on days 2–7 of drug administration, indicating that little correction or enhancement of the depressor effect occurred once the initial response to A-64662 was established. Infusion of the vehicle alone had no appreciable impact on MAP (Figure 2). In the vehicle-infused group, daily MAP values continued to approximate closely control levels, averaging in the range of 88±3 to 91±3 mm Hg. Thus, A-64662 administration produced a significant and sustained hypotension when compared with the vehicle-infused monkeys. One day after cessation of A-64662 infusion, MAP increased to 87±3 mm Hg and stabilized at control levels for the duration of the study. These results demonstrate that the hypotensive effects of A-64662 were completely reversible when administration of the compound was discontinued.

As shown in Figure 2, systolic and diastolic pressure decreased significantly from both control and the vehicle-infused group during A-64662 administration. Diastolic pressure tended to fall to a slightly greater extent than systolic pressure; however, pulse pressure was not significantly altered by drug administration. Heart rate ranged from 177±6 to 185±7 beats/min during the 4-day control period in the monkeys receiving A-64662. These values were in close agreement with the average heart rate observed during the same period in the vehicle-treated monkeys. Furthermore, no significant differences in heart rate occurred between the two groups throughout the study. On the fifth day of the study, heart rate in both groups averaged approxi-
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approximately 20 beats/min higher than the preceding day and a significant change from control was detected in the vehicle-treated monkeys. Thereafter, heart rate for the A-64662-treated monkeys stabilized at this higher level and ranged from 199±7 to 205±4 beats/min for the remainder of the study; somewhat greater daily fluctuations from 178±11 to 207±10 beats/min were evident in the vehicle-treated group.

The hypotensive response of the monkeys to the continuous infusion of A-64662 appeared to be submaximal at the dose that was used. The acute blood pressure response to an intravenous bolus of captopril or additional A-64662 was measured on the second and seventh days of the infusion, respectively, in five monkeys receiving A-64662 chronically. MAP was further reduced (p<0.05) from 78±6 to 65±6 mm Hg within 30 minutes after captopril administration and from 77±3 to 67±4 mm Hg with additional A-64662 before returning toward pretreatment values.

The temporal effects of the 7-day infusion of A-64662 on the renin-angiotensin-aldosterone axis in sodium-depleted monkeys are shown in Figure 3. PRA in the monkeys receiving A-64662 averaged between 55.3±9.8 and 60.4±11.4 ng Ang I/ml/hr during the 4-day control period. These values were similar to the control PRA values of the vehicle-treated group, which ranged from 50.9±14.2 to 57.6±15.7 ng Ang I/ml/hr. Infusion of A-64662 resulted in a significant inhibition of PRA as evidenced by the nearly 40-fold reduction of PRA to 1.4±0.8 ng Ang I/ml/hr after 1 day of A-64662 infusion. The inhibition of PRA by A-64662 was sustained throughout the drug infusion period as PRA values ranged between 0.6±0.4 and 1.9±1.0 ng Ang I/ml/hr on days 2–7. In comparison, PRA in the vehicle-treated monkeys did not change significantly from control during the 7-day infusion of the vehicle alone. During this period, PRA ranged from 39.0±6.1 to 63.0±19.1 ng Ang I/ml/hr in the vehicle-infused monkeys. Thus, PRA in the group receiving A-64662 was significantly lower than the vehicle group throughout the 7-day infusion of A-64662. Moreover, the difference in PRA between the treatment groups extended 1 day into the recovery period. After completion of A-64662 infusion, PRA increased progressively from 14.1±1.3 ng Ang I/ml/hr on the first day of the recovery period to 23.7±7.4 Ang I/ml/hr on the third day, although PRA remained below control levels for the entire recovery period. PRA in the vehicle-treated group declined during the recovery period to 25.9±8.0 ng Ang I/ml/hr on the final day of the study and a significant difference from control levels was detected.

A significant correlation (r=0.71, p<0.01) existed between PRA and plasma Ang II levels when control measurements from the monkeys receiving A-64662 and all values from the vehicle-infused group were examined. The plasma concentration of Ang II fell significantly below control during the infusion of A-64662 (Figure 3). The plasma Ang II concentration averaged 66.7±20.2 pg/ml in the control period and decreased over 80% to 12.4±3.3 pg/ml on the second day of A-64662 infusion. After 7 days of drug administration, plasma Ang II values averaged 26.4±6.5 pg/ml as five out of eight monkeys exhibited an increase on day 7 versus day 2 of A-64662 infusion. During the same period, plasma Ang II concentrations in the vehicle-treated group were 50.0±14.0, 46.0±8+14.4, and 41.8±18.3 pg/ml, respectively. A significant difference between the treatment groups was detected on the second day of the infusion period. On the final day of the recovery period, the plasma Ang II concentration in the vehicle-infused group was unchanged from control averaging 36.6±22.0 pg/ml, but a significant decrease from control remained in the monkeys receiving A-64662 as an average plasma Ang II concentration of 28.3±9.7 pg/ml was obtained.

The plasma aldosterone concentration decreased progressively during A-64662 infusion reaching a nadir of 31.6±9.6 ng% after 3 days of drug admin-
istration (Figure 3). Although this represented a greater than 60% decline in the PAC from control levels ranging from 79.8±17.4 to 90.1±14.4 ng%, a significant reduction existed only on the second and third days of A-64662 administration, and even then the PAC remained elevated well above sodium-replete levels. Furthermore, no significant differences in the PAC were evident between the treatment groups through the course of the study. PAC in the vehicle group ranged between 71.3±12.9 and 76.9±18.6 ng% during the control period and remained at similar levels until the recovery period when significant reductions from control were detected.

No significant changes in plasma cortisol concentrations were apparent within or between treatment groups throughout the study, including the period of A-64662 infusion. Measurements of plasma cortisol were performed on blood samples obtained every third day during the course of the study beginning on the second day of the control period. The plasma cortisol concentration ranged from 28.5±1.7 µg/dl to 31.6±2.5 µg/dl in the monkeys receiving A-64662 and from 21.9±1.3 to 31.8±4.2 µg/dl in the vehicle-infused group.

Urinary sodium excretion was constant, ranging from a low of 0.16±0.04 to a maximum of 0.41±0.12 meq/day, in the monkeys infused with A-64662 (Figure 4). In the vehicle-treated monkeys, sodium excretion varied between 0.11±0.04 and 0.56±0.14 meq/day. No significant differences in sodium excretion were observed between the two groups. Urinary potassium excretion was also unchanged in both groups during the study except for the transient reduction observed in the A-64662-infused group after 1 day of drug administration (Figure 4). The temporal pattern of plasma creatinine concentrations is also shown in Figure 4. Plasma creatinine concentrations were not altered significantly by drug infusion. However, in one monkey exhibiting a depressor response to A-64662 of greater than 20 mm Hg, a twofold to threefold elevation in plasma creatinine was observed for a period of 4 days before the levels returned to control for the remainder of the drug infusion period. Both treatment groups were observed to be in a relatively constant, but positive, sodium balance throughout the study (Table 1). Chronic administration of A-64662 had no observable effect on sodium excretion (Figure 4), and sodium intake was unchanged in either group during the study (data not shown). Thus, daily sodium balances were found to be in close agreement for both treatment groups, which in turn led to similar cumulative sodium balances as evidenced by the total of 10.4±1.6 meq for the monkeys treated with A-64662 compared with 11.1±2.3 meq for the vehicle-infused group over the 14-day study period. Plasma sodium and potassium concentrations are also shown in Table 1. No significant changes in plasma sodium concentration were detected in either group throughout the study. The plasma potassium concentration increased slightly albeit significantly, beginning on day 5 in both groups. No differences between the groups were evident at any time, however.

Plasma levels of A-64662 were determined from blood samples taken on days 2, 4, 6, and 7 of the continuous infusion. The plasma concentrations of A-64662 on these 4 days averaged 929±156, 821±115, 737±122, and 779±133 ng/ml, respectively.

**Discussion**

The present investigation demonstrates that the dipeptide renin inhibitor A-64662 is an effective hypotensive agent on a chronic basis when administered continuously to sodium-depleted monkeys. MAP decreased an average of 10-15 mm Hg almost immediately after A-64662 infusion was initiated and remained at nearly identical levels for a 7-day period. Accompanying this decrement in blood pressure, PRA was significantly inhibited, and plasma Ang II concentrations decreased below control levels. The effect of A-64662 on blood pressure was reversible, as evidenced by the return of MAP to
control levels within 1 day after cessation of A-64662 infusion. Demonstration of the chronic efficacy of A-64662 in blockade of the renin-angiotensin system and lowering of arterial pressure lends further support to the concept that renin inhibitors may serve as useful antihypertensive agents.

The renin-angiotensin system plays a major role in the regulation of normal blood pressure, which becomes increasingly dependent on the activity of this humoral axis during dietary sodium restriction and sodium deficiency. As exemplified by the seven monkeys followed before the study, PRA was stimulated nearly 14-fold higher than normal as a result of sodium depletion in the monkeys that were used for the present investigation. Normotensive sodium-depleted monkeys, therefore, provided a sensitive experimental model in which to evaluate the efficacy of A-64662. Dose selection of A-64662 was based solely on the consideration of providing an initial hypotensive effect of 10–20 mm Hg and avoiding any accumulation of excess inhibitor associated with highly saturating doses. Additionally, this degree of blood pressure reduction was easily detected in conscious monkeys seated in an upright position, but was not large enough to elicit any untoward effects. Despite the initially high level of plasma renin, infusion of A-64662 at a continuous rate of 0.01 mg/kg/min produced not only the desired hypotensive response, but also a striking inhibition of PRA to values well below that observed for sodium-replete monkeys, and in some animals PRA was undetectable throughout the A-64662 infusion period. After completion of A-64662 infusion, the recovery of MAP was rapid when compared with the recovery of PRA, which suggests that increased PRA was not solely responsible, at least initially, for the return of MAP to normotensive levels.

The greater than 95% inhibition of PRA that occurred during the chronic infusion of A-64662 was associated with a submaximal reduction in blood pressure as evidenced by the acute depressor activity of captopril or additional A-64662 beyond that already achieved by the continuous infusion of A-64662. This pattern of response is not unique, however. Previous studies have shown that further reductions in MAP occur despite undetectable levels of PRA with increasing doses of several renin inhibitors including A-64662. These findings have been postulated to result from the inhibition of some other renin pool important in the maintenance of arterial pressure such as vascular renin, or possibly renin in other tissue sites. In the present investigation, steady-state plasma concentrations of A-64662 quantified by HPLC averaged 817±132 ng/ml or 1.2±0.2 μM during the infusion of this compound in monkeys. This represents a concentration nearly 500 times in excess of the IC50 of A-64662 for monkey plasma renin at pH 7.4. This relation emphasizes the difficulties inherent in establishing a linear relation between the hypotensive response and PRA during the administration of a renin inhibitor as even minimally efficacious doses of A-64662 are quite likely to result in plasma levels well in excess of the IC50 and to generate near complete inhibition of PRA when quantified in vitro. Therefore, the results of the present study provide further evidence to suggest that other pools of renin may be important in blood pressure regulation or that the kinetics of the renin inhibitor interaction with plasma renin in vivo allows suffi-

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Values are mean±SEM. A-64662 group n=8; vehicle group n=6. *p<0.05 versus control.
cient Ang I formation despite drug concentrations well in excess of that needed to provide total renin blockade in vitro.

The observation that Ang II exerts a direct inhibition on renin release has led to concern that interruption of this negative feedback loop by renin inhibition could result in compensatory renin release sufficient to overcome the hypotensive effects of the initial blockade. In support of this concept, previous studies have found that immunoreactive plasma renin or PRA after dialysis increase as a result of acute or chronic renin inhibitor administration. In these same studies, however, PRA was consistently inhibited or nondetectable and more importantly, a sustained hypotension in sodium-deficient animals was observed in agreement with the results obtained in the present investigation. Thus, all of the evidence compiled to date indicates that a compensatory increase in renin release after a reduction of Ang II formation does not interfere with the hypotensive effect of renin inhibitors similar to the case in most patients with angiotensin converting enzyme inhibitors.

A reflex tachycardia was not observed in the monkeys during the initial 2-hour period after the start of A-64662 administration. Furthermore, on a chronic basis no changes in heart rate could be definitively associated with the hypotension induced by A-64662. However, the conclusion involving the long-term effects of A-64662 on heart rate must be tempered somewhat since both treatment groups exhibited increases in heart rate beginning on the first day of either vehicle or A-64662 administration. The reason for this increase is unclear, but conceivably, it may have obscured any chronic effect of A-64662 on heart rate. The lack of any measurable effect on heart rate despite a fall in blood pressure is consistent with the findings of other studies involving either renin inhibitors or angiotensin converting enzyme inhibitors. Ang II has been shown previously to potentiate activity of the sympathetic nervous system while also diminishing vagal tone. Therefore, a reduction in Ang II formation and attenuation of one or both of these interactions may account for the relative stability in heart rate concomitant with the hypotension that was observed in the present study.

A more specific index for blockade of the renin-angiotensin system is thought to be a reduction in the circulating levels of Ang II. Accordingly, a number of studies have focused on the effects of angiotensin converting enzyme inhibition and plasma Ang II concentrations, and although there is an abundance of evidence that plasma Ang II levels are reduced acutely, the chronic effects are controversial. Some investigators have found that blood pressure reductions are sustained even when Ang II formation appears to increase during chronic inhibition of angiotensin converting enzyme, but this has not been a systematic finding. The data indicate that, at least under certain experimental conditions, the hypotensive effects of angiotensin converting enzyme inhibitors occur in the absence of suppressed plasma Ang II concentrations. Unlike renin inhibitors, however, angiotensin converting enzyme inhibitors may reduce blood pressure also by virtue of their effects on other vasoactive hormones such as bradykinin and prostaglandins.

When using HPLC combined with radioimmunoassay, a 73% reduction from the vehicle group was observed in plasma Ang II levels after 2 days of A-64662 administration. This decrement paralleled the inhibition of PRA and the hypotension elicited by A-64662 and suggests that the reduction in MAP was brought about, at least partially, by reduced circulating levels of Ang II. Previously, other renin inhibitors have also been found to reduce plasma Ang II levels, but only the effects of acute administration have been reported. Unlike the initial effect, however, the present findings suggest that maintenance of the A-64662 hypotensive activity on a chronic basis was not necessarily related directly to changes in plasma Ang II levels. After 7 days of A-64662 administration, plasma Ang II levels, although remaining below control levels, increased over twofold from the nadir observed on the second day of A-64662 infusion, and a significant difference from the vehicle group no longer existed. The increase in Ang II was not accompanied by measurable changes in arterial pressure and only a small nonsignificant elevation (0.6±0.4 vs. 1.9±1.0 ng Ang I/ml/hr) in PRA was detected on day 7 when compared with day 2. The underlying mechanism whereby plasma Ang II levels increase during A-64662 administration is not readily apparent, but conceivably may represent enhanced synthesis through alternate enzymatic pathways or result from increased tissue renin activity inaccessible to the renin inhibitor. An equally perplexing result was observed after completion of A-64662 infusion when PRA gradually increased to levels exhibited by the vehicle group and arterial pressure returned to preinfusion values, whereas plasma Ang II concentrations remained essentially unchanged from the levels observed after 7 days of drug administration. It is not evident from the present study in what manner, if any, this observation is linked with the aforementioned rise in Ang II during A-64662 infusion. However, both observations suggest that the long-term hypotensive activity of A-64662 may not be a direct reflection of reduced circulating levels of Ang II since the chronic hypotension was not accompanied by completely sustained lower plasma concentrations of Ang II nor was the return of blood pressure to normotensive levels associated with a rise in circulating Ang II. Thus, the chronic hypotensive action of A-64662 may be mediated in part by inhibiting PRA and possibly an undefined mechanism related to renin inhibition.

In addition to its other actions, Ang II also stimulates the secretion of aldosterone. and previous studies have shown that under certain condi-
tions the PAC decreases during the administration of angiotensin converting enzyme inhibitors. 39 Although this effect appears to have less importance than blocking the other actions of Ang II, a putative reduction in the PAC and attenuation of its sodium-retaining actions offered a potential mechanism contributing to the maintenance of chronic hypotension brought about by renin inhibition. The PAC was reduced from control levels in the group treated with A-64662, but for only a 2-day period beginning on the second day of drug administration, and even then the concentration of aldosterone remained well above sodium-replete values. Moreover, when compared with the vehicle group, the PAC was not affected significantly by the continuous infusion of A-64662, unlike the effects observed on PRA and Ang II. Therefore, the results of the present investigation demonstrate that the PAC was reduced to only a small extent in response to A-64662, which suggests that a reduction of aldosterone secretion was most likely a negligible component in maintaining the hypotension of sodium-depleted monkeys during the 7 days of A-64662 administration.

The effects of renin inhibitors on aldosterone secretion have not been systematically examined. Therefore, it is unclear whether increased dosages of A-64662 would have elicited further decrements in the PAC in the present investigation. The heightened sensitivity of the adrenal glomerulosa to Ang II during sodium deprivation 41 may have been an important factor in maintaining a relatively constant stimulus for aldosterone secretion despite reduced plasma Ang II levels with A-64662. Alternatively, other aldosterone secretagogues, such as adrenocorticotropic hormone and potassium, may have played an increasingly important role in stimulating aldosterone secretion during A-64662 infusion. However, evidence for this was not forthcoming from either plasma cortisol concentrations, which remained unchanged, or from the plasma potassium concentrations that increased slightly but were within normal limits in both treatment groups during the course of the study. Additionally, the increase of plasma potassium in the vehicle group was actually simultaneous with a fall in the PAC. The explanation for the lack of a substantial effect of A-64662 on aldosterone secretion may also possibly reside in the limited access of the compound to adrenal renin. 29

Typically, most male cynomolgus monkeys with body weights similar to those used in the present study (3–6 kg) are continuing to grow and exhibit a positive sodium balance as they tend to retain 10–30% of ingested sodium when maintained on a normal sodium diet (unpublished observations). The results of the present study indicate, based on a percentage of the amount of sodium ingested, that sodium-depleted monkeys retained sodium even more avidly when maintained on a low sodium diet. Based on the magnitude of sodium depletion that was induced by furosemide administration before the study, nearly 50% of the estimated sodium deficit may have been regained by the monkeys in both treatment groups at the conclusion of the study. Thus, the fivefold reduction in the content of sodium in low versus normal sodium monkey chow alone was an insufficient stimulus for maintaining a completely stable sodium-depleted condition. Unfortunately, monkey chow with a still lower sodium content is less palatable and the daily caloric intake drops off dramatically.

Although PRA and plasma aldosterone were elevated well above normal sodium-replete values throughout the investigation, the partial restoration of sodium and fluid volume would account for the gradual reduction in activity of the renin-angiotensin-aldosterone axis that was evident in the vehicle-treated monkeys. It was probably also a major factor in determining the level of PRA achieved after the completion of A-64662 administration. PRA remained below control during the recovery period in the monkeys receiving A-64662. Nonetheless, the gradual accumulation of sodium cannot account for the discrepancy between the rate of recovery of arterial pressure as opposed to PRA with cessation of A-64662 infusion. The retention of sodium in the monkeys treated with A-64662 also did not affect the sensitivity of the hypotensive response to a detectable degree during the course of drug administration. This observation is not entirely unexpected in light of a previous study, which demonstrated that arterial pressure in dogs chronically treated with captoril also remained below control levels until dietary sodium intake was substantially increased.

Most evidence indicates that, with reductions in renal perfusion pressure, an intact renin-angiotensin system is necessary for autoregulation of glomerular filtration via Ang II efferent arteriolar constriction. 42 This mechanism is particularly apparent under conditions of sodium restriction and, thus, maintenance of the glomerular filtration rate (GFR) in the sodium-depleted monkeys was a special interest during the prolonged hypotension that resulted from blockade of the renin-angiotensin system by A-64662 administration. Although GFR determinations per se were not conducted, based on the lack of changes in the plasma creatinine concentration, GFR was not affected to a large extent by A-64662 administration when compared with either control levels or the vehicle-treated group. These results suggest that the depressor response to A-64662 was probably of insufficient magnitude in the present study to substantially reduce GFR and in turn adversely affect the regulation of sodium excretion through this mechanism.

Despite relatively large decreases in arterial pressure, which normally would tend to depress sodium excretion, no changes in the urinary excretion of sodium were detected in the monkeys infused with A-64662. Both treatment groups excreted sodium at nearly equivalent rates throughout the present investigation. This finding indicates that after blockade of
Ang II formation by renin inhibition, a normal balance between sodium intake and output was achieved at a lower than normal arterial pressure. The effect of A-64662 on sodium excretion essentially parallels previous observations obtained after administration of angiotensin converting enzyme inhibitors⁴⁰ and, therefore, appears to be a general response to blockade of the renin-angiotensin system. In opposition to the effects of lower renal perfusion pressure on sodium excretion, attenuation of the direct antinatriuretic actions of Ang II⁴⁵ would tend to promote the excretion of sodium by the kidney. Thus, the level of sodium excretion obtained during inhibition of renin may have been determined to a large extent by the interaction between these two pharmacological effects.

In summary, the continuous intravenous administration of the renin inhibitor A-64662 for a period of 7 days resulted in a sustained hypotension in sodium-depleted monkeys. Tolerance to the hypertensive activity of A-64662 was not evident and blood pressure was restored completely to normotensive levels when the infusion was discontinued. Finally, the hypotension induced by A-64662 was accompanied by a marked inhibition of PRA and a reduction in plasma Ang II concentrations.

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References


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K M Verburg, H D Kleinert, J R Kadam, M A Chekal, P F Mento and B M Wilkes

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