Comparison of arterial pressure and plasma ANG II responses to three methods of subcutaneous ANG II administration

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Kuroki MT, Fink GD, Osborn JW. Comparison of arterial pressure and plasma ANG II responses to three methods of subcutaneous ANG II administration. Am J Physiol Heart Circ Physiol 307: H670–H679, 2014. First published July 3, 2014; doi:10.1152/ajpheart.00922.2013.—Angiotensin II (ANG II)-induced hypertension is a commonly studied model of experimental hypertension, particularly in rodents. The dose of ANG II required to generate hypertension via the subcutaneous route is usually ~10-fold that required for the model generated via the intravenous route (4). The reported dose for rats in the literature ranges from 50 to 500 ng·kg−1·min−1, with a commonly used dose between 100 and 200 ng·kg−1·min−1 (4). The typical duration of ANG II administration is 2 wk (14, 15). The hypertensive response to chronic ANG II administration is often characterized by a gradual rise in pressure commonly referred to as a “slow pressor” response or the “auto-potentiating” effect of chronic ANG II administration (1, 2, 7, 8, 23). The severity of the slow pressor response and final blood pressure during chronic ANG II administration is dependent on both the dose of ANG II and impending level of dietary salt intake (20).

Our laboratory has been studying the neurogenic mechanisms of ANG II-induced hypertension in rats fed a high-salt diet (2% NaCl) using Alzet minipumps as the primary method of subcutaneous ANG II administration (150 ng·kg−1·min−1 for 2 wk) (14, 15, 21, 28). In our experience, the model generated using this method occasionally fails to demonstrate the slow pressor effect and sustained rise in pressure that is characteristic of the model. We have occasionally found that, in some animals subjected to the ANG II-salt protocol, pressure begins to decline gradually starting the second week of ANG II infusion, resulting in a blunting of the slow pressor response and reduced final blood pressure. There have been no distinguishable physical signs between rats that showed the normal progression in pressure versus those that displayed the latter response.

There are at least three explanations for the “nonresponder” profile, including 1) failure of the pump to maintain a constant flow rate over the 14-day protocol, 2) degradation of ANG II within the pump, or 3) failure of the rat to respond to ANG II (i.e., a true physiological nonresponder). For the present study,

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we focused our attention on pump performance as a contributing factor.

Until recently, the only implantable pump for rodents was the Alzet osmotic minipump (Durect, Cupertino, CA). However, another pump has recently become available: the iPrecio implantable pump. Unlike Alzet pumps, iPrecio pumps are miniaturized mechanical pumps that expel fluid from a reservoir using a motorized peristaltic rotor that is precisely controlled by an embedded microcontroller (26). A recent report (26) has shown that slight differences exist in the temporal profile of the pressor response to ANG II infusion using Alzet versus iPrecio implantable minipumps. Although rats in both Alzet and iPrecio groups displayed a slow pressor response to chronic 14 days of ANG II and the final level of pressure was comparable between the two groups, the initial rise in pressure was slightly blunted in rats in the Alzet group compared with the iPrecio group (26). This suggested the possibility that characteristics inherent to the Alzet pump could play a role in the unpredictable responses described above.

In the previously reported study, no other variables, such as plasma ANG II concentration, were measured to provide a possible explanation for the observed differences in the profile of ANG II-induced hypertension between Alzet and iPrecio groups. In addition, the observed differences were based on a comparison with a device that has just recently been introduced to the scientific community, making it difficult to assess whether these results could be generalized compared with other traditional methods of delivery, such as an external infusion pump. Furthermore, the reported study was based on a protocol using an undisclosed level of dietary salt. Our laboratory has shown that the neurogenic component of ANG II-induced hypertension is dependent on dietary salt intake, and our primary interest was to determine whether the different methods would impact the expression of the "neurogenic phase" that we have shown occurs during the later stage of ANG II infusion in rats that consumed a high-salt diet.

In this study, we compared the reproducibility of the ANG II-salt model of hypertension generated by subcutaneous administration of ANG II using three different delivery methods. We compared the differences in the blood pressure profile of ANG II-salt hypertension generated using Alzet, iPrecio, and Harvard infusion pumps attached to an implanted subcutaneous infusion catheter and tested whether any differences between groups could be explained by differences in the change of plasma ANG II levels over time.

**METHODS**

Experiments were performed in conscious, chronically instrumented rats in accordance with National Institutes of Health guidelines. All acute and chronic experimental procedures were conducted after approval by the Institutional Animal Care and Use Committee of the University of Minnesota.

**Animal Use and Care**

Male Sprague-Dawley rats from Charles River Laboratories (Wilmington, MA) weighing 200–250 g upon transfer to our facility were used in this study. Depending on the protocol (see below), rats were kept on a regular diet (Lab Diet 5012) or switched to a special diet with variable NaCl content (Research Diets, New Brunswick, NJ). Animals were housed at 2 rats/cage in a 12:12-h light-dark cycled room (8:30/20:30 cycle). Animals were allowed to acclimate for at least 1 wk before surgery.

**Experimental Protocol**

Experiment 1: establishment of the physiological range of endogenously generated plasma ANG II. The protocol for this experiment is shown in Fig. 1, top. Rats were randomly assigned to three groups based on dietary NaCl content: 0%, 0.1%, or 0.4%. Rats were then subjected to additional interventions to stimulate ANG II release, and blood was collected again 7 days later. This was used for the subcutaneous delivery of a jugular venous catheter for blood collection as described below.

Venous blood was collected from all three groups 7 days after surgery. Two of the groups (0% and 0.4% NaCl) were then subjected to additional interventions to stimulate ANG II release, and blood was collected again 7 days later. In rats fed the 0% NaCl diet, the loop diuretic furosemide (F4381, Sigma-Aldrich, St. Louis, MO), a known stimulant for renin release and subsequent increase in plasma ANG II, was administered (50 mg/kg ip), and blood was collected ∼3 h later. For the 0.4% NaCl diet, rats were subjected to another stimulus for renin release, 48-h water deprivation, 5 days after the initial blood collection, and blood was then collected at the end of the 48-h period. Plasma ANG II was assayed using a commercial ELISA kit (described below).

Experiment 2: comparison of the effect of three infusion methods on plasma ANG II and arterial pressure during ANG II-salt hypertension. The protocol for this experiment is shown in Fig. 1, bottom. Rats were fed the 2% NaCl diet, given distilled water ad libidum, and randomly assigned to one of three groups based on the method of ANG II delivery: 1) Alzet osmotic minipump (2ML2, Durect), 2) iPrecio microinfusion pump (SMP-200, Primetech, Tokyo, Japan), or 3) Harvard syringe pump (model 935, Harvard Apparatus, South Natick, MA). Rats were chronically instrumented with a Data Sciences (St. Paul, MN) pressure transmitter and a jugular venous catheter. For rats in the Harvard group, a catheter was tunneled subcutaneously over the right flank area, exteriorized, and connected to a single channel hydraulic swivel (model 375/22PS, Instech Laboratories, Plymouth Meeting, PA). This was used later for the subcutaneous delivery of ANG II. All other rats were tethered to an in-house-made swivel. Rats were given 7–10 days to recover from surgery. The surgical procedure is described in detail below.

The study protocol consisted of 4 days of control followed by 14 days of ANG II infusion. For the Alzet group, a second surgery was performed on the fifth day of the protocol for minipump implantation as previously described (15). In all groups, the entire volume of the ANG II infusate required for the protocol was made by dissolving ANG II (A9525, Sigma-Aldrich) in physiological saline and stored at room temperature. ANG II was delivered at an infusion rate of 5 μl/h and dosed at 150 ng·kg⁻¹·min⁻¹ based on body weight on control day 1. Blood was sampled on control day 1 and on days 3, 7, and 14 of ANG II for measurement of plasma ANG II concentrations (described below). Arterial pressure data were collected using acquisition software from Data Sciences (DataQuest ART Acquisition) as previously described (15).

Experiment 3: establishing the relationship between the estimated flow rate of Alzet pumps and steady-state mean arterial pressure in ANG II hypertension. Based on the outcome of experiment 2 (see RESULTS), an additional experiment was performed to assess the relationship between the estimated flow rate of Alzet pumps and the steady-state response of arterial pressure to ANG II administration. ANG II hypertension was generated using Alzet pumps using the same protocol described in experiment 2 with one exception: experiments were conducted in rats that consumed either a low-salt (0.1% NaCl) diet (n = 17) or a high-salt (2.0% NaCl) diet (n = 20). We included this treatment to determine whether pump performance was affected by arterial pressure, since the hypertensive response to ANG II is less in rats on a low-salt diet (15). Rats were instrumented with a Data Sciences pressure transmitter, but no jugular venous catheter was implanted as no plasma was sampled in this experiment. At the end of the 14-day infusion period, pumps were removed, and the
residual volume was measured by careful withdrawal of fluid into a 1-ml syringe. This residual volume was used to estimate the average 24-h infusion rate for Alzet pumps over the 14-day infusion period and compared with mean arterial pressure (MAP) averaged from days 10–13 of ANG II infusion.

Surgical Procedures

Surgery was performed using aseptic techniques under isoflurane anesthesia (2.5% isoflurane in 100% O2). After induction, rats were given atropine (0.2 mg/kg ip, Baxter, Deerfield, IL) for reduction of salivary and bronchial secretions, preoperative antibiotic prophylaxis [gentamicin (0.05 ml im), Hospira, Lake Forest, IL], preoperative pain relief [ketoprofen (5 mg/kg sc), Fort Dodge Animal Health, Overland Park, KS], and placed on a heated surgical bench.

The jugular vein was exposed via a supraclavicular skin incision, and a catheter consisting of a 6-cm intravascular Silastic tip (0.02-in. inner diameter / 0.037-in. outer diameter, Silastic Laboratory Tubing, Dow Corning, Midland, MI) connected to Tygon tubing (0.02-in. inner diameter / 0.06-in. outer diameter, Tygon Micro-Bore tubing S-54-HL, Saint-Gobain Performance Plastics, Akron, OH) was inserted 3 cm toward the right atrium. The catheter was sutured in place, tunneled subcutaneously through the chest, and then secured over the pectoral musculature before being exteriorized through a dorsal incision over the scapulae.

Rats in experiment 2 were also instrumented with a Data Sciences pressure transmitter (TA11PA-C40) for measurements of arterial pressure as previously described (15). In the Harvard group, a catheter (23-gauge, Tygon) was implanted subcutaneously such that the tip of the catheter was positioned over the animal’s right lower flank. In the iPrecio group, an iPrecio minipump filled with physiological saline was implanted subcutaneously over the animal’s right flank. The tip of the pump’s infusion catheter was tunneled across to the animal’s left flank. The body of the minipump was secured to the underlying subcutaneous tissue via the pump’s suture holes.

The catheters were exteriorized through a skin incision over the scapulae and anchored to the underlying subcutaneous tissue using a circular surgical polyester mesh (PETKM14002, Textile Development Associates, Surgical Mesh Division, Brookfield, CN), which was implanted subcutaneously and sutured to the skin upon closure of the incision. The exteriorized catheters were threaded through a stainless steel spring used for tethering the rat to a custom-made or commercial single channel hydraulic swivel mounted above their cage.

Rats were given a minimum of 7 days to recover from surgery. Ketoprofen (5 mg/kg sc, Fort Dodge Animal Health) was administered daily for 3 days postsurgery for pain management. Jugular venous catheters were flushed daily with lock solution (50 U/ml heparinized saline, Hospira) to ensure patency.

Indirect Assessment of Pump Flow Rates

The iPrecio pumps were programmed to deliver ANG II at a rate of 5 μl/h using the accompanying PC software and programming device. The infusion rate for iPrecio pumps was experimentally verified in a subset of eight rats in experiment 2 from the refill volume and time interval between refills. Based on the length of the protocol and the 900-μl reservoir, four refills were required over the course of the experiment. The Harvard syringe pump was calibrated to an infusion rate of 5 μl/h before the study based on accurate measurements of the syringe diameter and displacement rate of the plunger. The plunger displacement rate over a 12-h period was checked periodically during the experimental protocol to ensure the proper infusion rate of ANG II. The infusion rate for the Alzet pumps was not determined for the
Blood Collection and Separation of Plasma

Whole blood (0.35 ml) was collected from the jugular venous catheter over a period of 30–60 s into a syringe containing 15 μl of an inhibitor cocktail. The inhibitor cocktail consisted of a mixture of EDTA (125 mM, E4884, Sigma-Aldrich), pepstatin-A (20 μg/ml in methanol, 77170, Sigma-Aldrich), 1,10-phenanthroline (8 μg/ml, 131337, Sigma-Aldrich), enalaprilat (80 μg/ml in 50% ethanol, E9658, Sigma-Aldrich), 4-amidinophenylmethanesulfon fluoride hydrochloride (800 μg/ml, A6664, Sigma-Aldrich), and 2-mercapto-ethanol (2%, M6250, Sigma-Aldrich) based on a previously described protocol (16). The inhibitor cocktail was prepared fresh before each sample collection from previously prepared stock solutions. Blood was chilled on ice immediately after collection. After blood collection, 1 ml of physiological saline was administered intravenously, and the catheter was filled with lock solution. Blood was centrifuged at 2,000 g for 10 min. Plasma was collected and recentrifuged at 16,000 g for an additional 10 min and stored at −80°C until assayed for ANG II. On average, 180–200 μl of plasma were recovered using this technique.

ANG II assay

Before the assay, plasma was thawed, acidified with 1% trifluoroacetic acid (TFA; 302031, Sigma-Aldrich) and centrifuged at 16,000 g for 10 min. It was then purified through a C18 column (Y1000 SEP-COLUMNS, Peninsula Laboratories, San Carlos, CA), washed for 10 min. It was then purified through a C18 column (Y1000 SEP-COLUMNS, Peninsula Laboratories, San Carlos, CA), washed for 10 min. Plasma was collected and recentrifuged at 16,000 g for an additional 10 min and stored at −80°C until assayed for ANG II. On average, 180–200 μl of plasma were recovered using this technique.

Data Analysis and Statistics

Arterial pressure and heart rate (HR) data for individual animals were averaged over a 24-h period. Duplicate absorbance readings from plasma samples were averaged and converted to units of picograms per milliliter based on a standard provided with the kit. Grouped data are shown as means ± SE. The effect of dietary NaCl on plasma ANG II levels was analyzed by one-way ANOVA followed by multiple comparisons (Holm-Sidak) versus control (0.4% NaCl). The effect of furosemide and 48-h water deprivation on 0% and 0.4% NaCl-fed rats was analyzed by a paired t-test. The effect of infusion method on plasma ANG II, MAP, and HR was analyzed by repeated-measures ANOVA followed by a Holm-Sidak multiple-comparisons test when appropriate. All aforementioned analysis were performed in SigmaPlot (version 11, Systat Software, Richmond, CA). To determine the relationship between plasma ANG II and MAP in experiment 2, a linear regression was performed between the change in MAP and change in plasma ANG II compared with control levels using JMP Pro (version 10, SAS Institute, Cary, NC). The relationship was assessed separately for days 3, 7, and 14 of ANG II. The degree of correlation was assessed using the Pearson correlation coefficient calculated in JMP Pro.

RESULTS

Experiment 1: Establishment of the Physiological Range of Endogenously Generated Plasma ANG II

Plasma ANG II responses to various stimuli showed physiologically consistent trends (Fig. 2). Compared with plasma ANG II in rats fed a normal salt (0.4% NaCl) diet (9 ± 2 pg/ml), plasma ANG II increased approximately fivefold to 50 ± 10 pg/ml when dietary NaCl content was lowered to 0.1%. There was no further rise in plasma ANG II levels (50 ± 6 pg/ml) when dietary NaCl content was further lowered to a minimum NaCl diet (0% NaCl). Acute furosemide injection to 0% NaCl-fed rats to stimulate renin release and plasma ANG II production caused an ~3 fold increase in plasma ANG II levels to 170 ± 30 pg/ml compared with baseline and an ~18-fold increase compared with plasma levels from 0.4% NaCl-fed rats. Water deprivation for 48 h in 0.4% NaCl-fed rats caused an approximately threefold increase in plasma ANG II levels to 31 ± 3 pg/ml. The plasma ANG II level in

![Graph showing plasma ANG II levels in response to physiological salt loading, water deprivation, and pharmacological salt depletion. Left: results from experiment 1. Data for the 2% NaCl diet group is the control period plasma ANG II level from experiment 2 averaged across all three groups of rats (i.e., Alzet, Harvard, and iPrecio groups). *Statistically significant effect after furosemide or water deprivation within the given dietary NaCl group. †Statistically significant increase in plasma ANG II levels during chronic infusion of ANG II (150 ng·kg⁻¹·min⁻¹ sc) using Harvard (n = 9), Alzet (n = 12), or iPrecio (n = 10) pumps. The plotted plasma ANG II level represents the average from days 3, 7, and 14 of ANG II infusion. There were no between-group differences. ‡Statistically significant increase in plasma ANG II levels during ANG II infusion in Harvard, Alzet, and iPrecio groups compared with their respective control levels. n.s., Not significant. Statistical significance was set at P < 0.05 or less with post hoc comparisons using the appropriate correction for the significance level. Error bars are SEs.](http://ajpheart.physiology.org/.)
Experiment 2: Comparison of the Effect of Three Infusion Methods on Plasma ANG II and Arterial Pressure During ANG II-Salt Hypertension

Arterial pressure and HR responses to ANG II administration.
The three methods for chronic subcutaneous ANG II infusion resulted in a subtle difference in the initial changes in MAP but marked differences in the final week of ANG II infusion. MAP at day 14 of ANG II was highest in the iPrecio group (156 ± 5 mmHg) followed by the Harvard (140 ± 5 mmHg) and Alzet (122 ± 4 mmHg) groups. The largest single day rise in MAP occurred on day 1 of ANG II infusion. The increase in MAP (compared with baseline) on day 1 of ANG II was highest in the iPrecio group (24 ± 2 mmHg) followed by the Alzet group (17 ± 2 mmHg) and then the Harvard group (11 ± 1 mmHg). Pressure rose gradually after this first day. Despite differences in the day 1 ANG II MAP response, the slow pressor response was similar between iPrecio and Harvard groups. The slow pressor response to ANG II infusion was calculated by subtracting MAPs on days 1–14 of ANG II from MAP on day 1 of ANG II. This slow pressor response was markedly blunted in the Alzet group, which was due, in part, to the decrease in MAP that occurred in a subset of rats starting around day 8 of ANG II. The slow pressor response from day 1 onward contributed 33 ± 3, 29 ± 3, and 6 ± 3 mmHg of the total change in pressure seen on day 14 of ANG II in the iPrecio, Harvard, and Alzet groups, respectively.

The HR response to chronic ANG II infusion (Fig. 3, bottom) was also different among the three groups. HR decreased in all groups during the first week of ANG II. The peak drop in HR occurred on days 3 and 4 of ANG II for the Alzet and iPrecio groups, respectively, and on day 8 of ANG II for the Harvard group. After this nadir, HR tended to increase in all groups and was most noticeable in the iPrecio group. HR on day 14 of ANG II was 420 ± 10, 395 ± 8, and 402 ± 7 beats/min in the iPrecio, Harvard, and Alzet groups, respectively.

Plasma ANG II responses to ANG II administration. Figure 4 shows plasma ANG II during control and on days 3, 5, 7, and 14 of ANG II infusion and the corresponding values for
arterial pressure in the three groups. Plasma ANG II during control was not statistically different between groups. Although mean plasma ANG II levels during ANG II administration for the entire infusion period (Fig. 2, right), expressed as the combined average among days 3, 7, and 14 of ANG II, were not statistically different between the three groups (59 ± 8, 62 ± 7, and 71 ± 8 pg/ml for the Alzet, Harvard, and iPrecio groups, respectively), there were differences in the temporal profile of plasma ANG II during ANG II infusion between the three groups (Fig. 4, top). Rats in the Alzet group showed a marked peak in plasma ANG II on day 3 of ANG II administration followed by a marked decline on days 7 and 14. In both Harvard and iPrecio groups, this peak in plasma ANG II did not occur until day 7 of ANG II. Similarly to the Alzet group, plasma ANG II in the Harvard group declined markedly on day 14 of ANG II; however, day 14 plasma ANG II levels in the iPrecio group remained near day 7 levels.

Correlation of the changes in plasma ANG II and arterial pressure in all rats. The data shown in Fig. 4 were reanalyzed to determine the relationship between changes in plasma ANG II induced by exogenous administration of ANG II and the response of arterial pressure (Fig. 5). This correlation was based on combined data from the Alzet, iPrecio, and Harvard groups. On day 3 of ANG II administration, despite a wide range of increases of plasma ANG II, the slope of the relationship between the increase in plasma ANG II and the increase in MAP was flat and statistically insignificant. Although the slope of this relationship tended to increase on day 7 of ANG II, there was still no statistically significant correlation. However, by day 14 of ANG II, there was a marked increase in the slope of the direct relationship between changes in plasma ANG II and increases in MAP, and the correlation was statistically significant.

Experiment 3: Establishing the Relationship Between the Estimated Flow Rate of Alzet Pumps and Steady-State MAP in ANG II Hypertension

In a separate experiment, we established the relationship between the residual volume of ANG II infusate in explanted Alzet minipumps and MAP after 14 days of ANG II infusion in rats on low- and high-salt diets (Fig. 6). The pump infusion rate was estimated by subtracting the residual volume from the
filling volume divided by the total hours the pump was implanted. Based on the nominal infusion rate of the pump (5 μl/h), the residual volume should be 0.32 ml after 14 days of infusion. However, the residual volume ranged from 0.54 to 0.77 ml. This reflects a range of infusion rates from 4.34 to 3.62 μl/h, respectively. Estimated infusion rates for rats on a high-salt diet (3.97 ± 0.03 μl/h) and low-salt diet (4.02 ± 0.04 μl/h) were not statistically different. MAP was statistically higher in high-salt diet-fed rats (142.6 ± 5.0 mmHg) compared with low-salt diet-fed rats (114.6 ± 2.5 mmHg). Not only were these infusion rates variable, but they were also below 5 μl/h, as suggested by Alzet, with an overall average value of 3.99 μl/h, which translates to a 21% lower rate of ANG II administration over a 14-day period than predicted.

**Indirect Assessment of Pump Flow Rates**

We also estimated the infusion rate of iPrecio pumps in eight rats based on filling volumes over the protocol. The reservoir was refilled four times over the course of the experiment, twice with saline during the time between instrumentation and the beginning of the ANG II period and twice during the ANG II infusion period. Figure 7 shows the estimated flow rates in eight rats based on the four refill times as well as an overall average. Based on this assessment, the mean flow rate was 4.6 μl/h, which was 6% slower than predicted, which translated to a 6% lower rate of ANG II administration over a 14-day period than predicted.

**DISCUSSION**

Our group has years of experience using Alzet minipumps to study the ANG II-salt model of hypertension (12, 13, 14, 15, 21, 28). During this time, we found that, in random groups of animals, the arterial pressure response to ANG II begins to decline gradually starting at the second week of ANG II infusion. This results in a blunting of the slow pressor response and reduced final blood pressure, making comparison and interpretation of cardiovascular responses in the treatment groups difficult.

We hypothesized that the variability in the slow pressor response of the ANG II-salt model was mainly due to factors inherent in the performance of Alzet minipumps. Therefore, in the present study, we compared the arterial pressure profile of ANG II-salt hypertension generated using Alzet minipumps, iPrecio implantable pumps, and an external infusion pump (Harvard) connected to a subcutaneously implanted catheter. We also measured plasma ANG II levels during control and on days 3, 7, and 14 of ANG II, as we reasoned that differences in the pump’s ability to maintain the expected delivery rate of ANG II would be a main underlying cause for any between-pump differences.

**Response of Plasma ANG II to Exogenous Subcutaneous Infusion of ANG II: Comparison With Endogenously Generated ANG II**

Using a commercially available ANG II ELISA, plasma ANG II concentrations in rats fed a “regular” salt diet (~0.4% NaCl) were comparable to values reported in the literature, which ranged between 11 and 50 pg/ml in normal rats (5, 9, 25, 30). In rats fed a 2% NaCl diet, plasma ANG II levels did not decrease any further compared with 0.4% NaCl-fed rats, despite the fivefold increase in dietary salt intake. The absence of a statistically distinguishable difference could have been due to a limitation in the sensitivity of the assay, but it nevertheless suggests that rats fed a regular diet are at the tail end of a plasma ANG II versus salt intake response curve. In general, when all three methods of subcutaneous ANG II administration at 150 ng·kg⁻¹·min⁻¹ were examine, plasma ANG II concentration in 2% NaCl-fed rats increased approximately fivefold. This response was well within the physiological range of ANG II compared with levels seen in salt-depleted rats fed a 0.1% or minimum (0%) NaCl diet. Even at peak levels observed on day 3 of ANG II in Alzet rats, plasma ANG II values were within a physiologically attainable range as values were below those seen in salt-depleted rats acutely treated with furosemide.

In comparison, plasma ANG II concentrations reported by others in ANG II-induced hypertension ranged from 26 pg/ml to 400 pg/ml in rats given ANG II at 100 ng·kg⁻¹·min⁻¹ (25), 34 pg/ml for 175 ng·kg⁻¹·min⁻¹ (5), 47–157 pg/ml for 200 ng·kg⁻¹·min⁻¹ (5, 9), 79 pg/ml for 350 ng·kg⁻¹·min⁻¹ (5), and 101 pg/ml for 500 ng·kg⁻¹·min⁻¹ (5). A recent study (10) reported no change in plasma ANG II using the same dose used in this study but found a fourfold increase when 500 ng·kg⁻¹·min⁻¹ was administered. In most of these cases, plasma ANG II levels during ANG II infusion were not statistically distinguishable from control levels. In those that reported a statistically significant change, there was twofold change from control levels for 100 ng·kg⁻¹·min⁻¹ (25), threefold change from control levels for 200 ng·kg⁻¹·min⁻¹ (30), and sixfold change from control levels for 500 ng·kg⁻¹·min⁻¹ (5). Although the rise in plasma ANG II measured during ANG II-salt hypertension was higher in our study for the given delivery rate of ANG II, direct between-study comparisons are difficult to make due to differences in the level of dietary salt used in this (2% NaCl) and other studies (likely ~0.4% NaCl) and the high degree of variability in the reported values, both within and between

![Fig. 7. Predicted volume flow rate of iPrecio pumps based on refill volumes. Predicted volume flow rates for individual rats based on the refill volume at four time points during the protocol are shown. For rat 8, data points are overlapping. Values are means ± SE based on all measurements.](http://ajpheart.physiology.org/content/doi/10.1152/ajpheart.00922.2013)
studies. Variations between studies are likely due to differences in the ANG II assay.

Finally, it is useful to compare our results with those of the classic study of Brown and colleagues (2), who measured arterial pressure and plasma ANG II responses to continuous intravenous ANG II infusion at a dose of 20 ng·kg⁻¹·min⁻¹ for 7 days using an external infusion pump. This intravenous dose increased plasma ANG II sixfold above normal with an increase in MAP of ~50 mmHg. These results are similar to what we observed in the iPrecio group in the present study.

Does Pump Performance Explain the Differences in Arterial Pressure Responses to ANG II Administration Using Different Delivery Methods?

Several findings in this study suggest that differences in the arterial pressure response to ANG II administration between groups, particularly at steady-state conditions, are directly linked to the ability of the delivery method to maintain stable plasma ANG II concentrations over the duration of the protocol. First, arterial pressure on the final day of the study was highest in rats in the iPrecio group and lowest in rats in the Alzet group. This corresponded to the plasma concentration of ANG II at that time point. Second, of the three delivery methods used, iPrecio pumps resulted in the most stable plasma ANG II concentration over the duration of the study. By day 14 of ANG II, plasma ANG II and arterial pressure were higher in the iPrecio group than in the other two groups. In contrast, plasma ANG II was the most variable in rats in the Alzet group. This group had the highest concentration of all groups on day 3 of ANG II administration but the lowest concentration by the end of the protocol. Fourth, based on indirect assessment of pump flow rates over the 14-day administration period, Alzet pumps functioned at 79% of the nominal flow rate compared with 94% for the iPrecio pumps. Fifth, the entire volume of ANG II infusate was made up before the protocol at the same volume and concentration in all groups. Therefore, consistent with a previous report (9), differences in arterial pressure and plasma ANG II responses are not likely to be explained by degradation of ANG II within the pump. Based on these findings, we conclude that the single greatest determinant to the variability of the ANG II-salt model of hypertension in which ANG II is administered by Alzet osmotic minipumps is variability in pump performance.

Griffin and colleagues (9) have suggested that within-study variability in plasma ANG II concentration in models of ANG II-induced hypertension, specifically when Alzet pumps are used, could potentially be due to intermittent pumping that result in unpredictable fluctuations in plasma ANG II. In the same report (9), it was proposed that “the manufacturer notes that subcutaneous pumps may infuse at 10–15% below the in vitro rate” and therefore the dose of ANG II needs to be adjusted accordingly.

We found that rats in the Alzet group had a marked spike in plasma ANG II on day 3 of ANG II administration, which rapidly trailed off over the subsequent period of ANG II administration. This pattern was not observed in rats in which ANG II was administered by a pump in which the flow rate was mechanically fixed (rats in the iPrecio and Harvard groups). It may be that for the Alzet pumps, there is an initially higher flow rate (11) that corresponds with osmotically driven compression of the pump bladder, and this rate then decreases over time. This may be one explanation for the nonresponder profile of the rats in the Alzet group, a rapid fall off in pump flow rate that does not occur with a “mechanical” pump.

Compared to the previously published “pilot” study (26), which showed a slight pump-dependent difference in the initial profile of MAP during ANG II-induced hypertension, differences between Alzet and iPrecio pumps in the present study were larger in magnitude and especially pronounced during the second week of ANG II infusion. Based on the level of the slow pressor response, measured in terms of the day-to-day change in pressure averaged over days 2–14 of ANG II, the final level of MAP, and the variability in those values, the iPrecio group displayed the most “robust” ANG II-salt hypertension phenotype during the 2-wk protocol. The Harvard group had an intermediate final level of MAP but a similar slow pressor response to the iPrecio group. The Alzet group had the lowest final level of MAP and a significantly blunted slow pressor response due, in part, to a gradual drop in MAP in a subset of rats during the second week of ANG II infusion.

There were notable between-pump differences in the day 1 level of MAP, which ultimately impacted the final level of MAP at the end of the 2-wk infusion protocol. Day 1 MAP in the Harvard group was lowest among the three groups. One likely explanation for this lower level of MAP is the difficulty in adjusting the infusion rate of the pump to match that of Alzet and iPrecio pumps. Although the Harvard pump was calibrated to an infusion rate of 5 µl/h based on accurate measurements of syringe diameter and displacement rate of the plunger, it is possible that compliance within couplings of the long infusion line and undetectable leakage in the hydraulic swivel from elevated back pressure due to material buildup at the tip of the implanted catheter or a kink at the site of exteriorization caused a lower volume to be delivered compared with the expected rate.

Although differences in day 3 plasma ANG II levels between Harvard and iPrecio groups do not reflect a lower ANG II delivery rate in the Harvard group, baroreflex and negative feedback effect of ANG II on renin secretion could have resulted in lower plasma ANG II levels in the iPrecio group, thus masking the initial effect of differences in the infusion rate. This uncoupling of plasma ANG II levels and MAP is also evident in day 3 values in rats in the Alzet group compared with the iPrecio group. Although ANG II levels were significantly higher in the Alzet group, there were no significant differences in MAP compared with the iPrecio group. One possibility is a higher initial pumping rate in Alzet pumps, as our protocol has a short preequilibration step (~2–3 h) and no warming step before pump implantation, both of which have been reported to affect initial pump performance when used in acute settings (11). The lower day 1 change in MAP compared with the iPrecio group makes this scenario unlikely; however, it is possible that accelerated pumping is delayed.

Differences in Day 1 MAP Response to ANG II Do Not Predict the Magnitude of the Subsequent Slow Pressor Response

If differences in the infusion rate of ANG II between groups were responsible for differences in the increase of MAP over time and the infusion rate was constant, one would predict
these changes should appear on the first day of ANG II administration and remain for the duration of the protocol. However, this was not the case. Even though the MAP response on day 1 of ANG II administration was lower in the Harvard group compared with the iPrecio group, the subsequent rate of rise of MAP over time was the same. On the other hand, the MAP response on day 1 in the Alzet group was similar to that in the Harvard group, but the subsequent rate of rise of MAP was significantly less. This suggests that the blunting and disappearance of the slow pressor response after day 7 of ANG II in the Alzet group is due to problems intrinsic to the use of Alzet pumps in this particular context. There are several explanations for this result. A gradual decrease in the pumping rate or a reduction in the effective concentration of ANG II in the infusate due to degradation within the pump could have resulted in a gradual reduction in the delivered dose of ANG II. Alternatively, it has been proposed that fibrotic changes or increased degradation of ANG II secondary to an inflammatory reaction around the site of pump implant result in reduced ANG II absorption (4). Although we did not investigate these possibilities, we reasoned that either scenario could have resulted in measurable changes in plasma ANG II levels paralleling changes in arterial pressure. Consistent with this concept, there was a downward trend in plasma ANG II levels in rats in the Alzet group, as reflected by the significantly lower plasma ANG II levels on days 7 and 14 of ANG II after its peak on day 3.

Physiological Implications

The primary objective of this study was to determine the extent to which performance characteristics of two implantable minipumps determined the stability and reproducibility of a widely used rodent model of hypertension. However, this study also generated potentially significant findings that may provide new insights into the mechanisms of ANG II-salt hypertension.

Collectively, our experiments have shown that the “early phase” of ANG II-salt hypertension is mediated by “non-neurogenic” actions of ANG II, most likely direct vasocostriction. However, beginning 5–7 days after the initiation of ANG II administration, the model becomes increasingly neurogenically driven over time (19). Indeed, we (22) have recently reported that intracerebroventricular infusion of the Na$^+$ channel blocker benzamil has no effect on the early phase but entirely normalizes pressure during the late phase. These experiments suggest that the mechanisms mediating the hypertensive response during the early and later phases of ANG II-salt hypertension are different. However, it is not clear at this point what the specific “dose-response” relationships are for plasma ANG II and arterial pressure during the early (direct actions of ANG II) and late (neurogenic actions of ANG II) phases of ANG II-salt hypertension.

Based on data generated in this study, we were able to closely examine the correlation between increases in plasma ANG II and MAP during both the early and late phases of ANG II-salt hypertension (Fig. 5). Interestingly, there was no correlation between these variables during the first week of ANG II administration. However, a relationship between plasma ANG II and MAP emerged at 14 days of ANG II. As we previously hypothesized (20), the initial hypertensive response to the vasoconstrictor actions of ANG II in the early phase is most likely buffered by the arterial baroreceptor reflex. However, as the baroreflex adapts over time, the synergistic actions of dietary salt and circulating ANG II on central sympathoexcitatory pathways are unopposed, and the neurogenic phase is more strongly expressed. Although data from the present study support this hypothesis, further studies are needed.

Summary and Perspectives

ANG II-induced hypertension has been a popular model due to its relative ease to generate a hypertensive phenotype. However, comparing results between laboratories are difficult due to the variety of protocols in use, ranging from differences in the dose of ANG II, the route of ANG II administration, and the level of dietary salt. The findings in this study show that Alzet pumps may add another source of variability due to characteristics inherent to the pump. The use of mechanical infusion devices, either implantable (iPrecio) or external (syringe pump), may remove this pump-dependent source of variability and result in a more reproducible and consistent ANG II-induced hypertension phenotype.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: M.T.K. and J.W.O. conception and design of research; M.T.K. performed experiments; M.T.K. and J.W.O. analyzed data; M.T.K., G.D.F., and J.W.O. interpreted results of experiments; M.T.K. prepared figures; M.T.K., G.D.F., and J.W.O. edited and revised manuscript; M.T.K., G.D.F., and J.W.O. approved final version of manuscript.

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