Low-Dose Angiotensin II Enhances Pressor Responses Without Causing Sustained Hypertension

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Abstract—Subpressor doses of angiotensin II (SP-Ang II) cause a slow increase in blood pressure in rats as assessed by tail cuff plethysmography (TCP), reflecting either sustained hypertension or an exaggerated pressor response to diverse stimuli. We examined whether subpressor doses of Ang II enhance blood pressure responses to simple stress (handling of trained rats for TCP). We implanted telemetry in Sprague-Dawley rats. After 10 days of recovery and TCP training, we implanted osmotic minipumps with either SP-Ang II (50 ng/kg per minute) or vehicle, and then measured systolic blood pressure continuously in unrestrained rats for 13 days. We also recorded telemetry readings while obtaining TCP measurements every 2 days. SP-Ang II increased blood pressure from 134±19 to 159±22 mm Hg by TCP, which matched the simultaneous telemetry readings of 131±20 to 154±25 mm Hg. In contrast, SP-Ang II did not change the blood pressure in the unrestrained rats (measured with continuous telemetry: 124±2 versus 127±1 mm Hg). The blood pressure in the control rats did not change in the unrestrained state (125±3 versus 128±5 mm Hg on days 0 and 12, respectively), and only slightly increased during TCP (11±5 and 6±4 mm Hg by TCP and simultaneous telemetry, respectively; P=NS). In summary, SP-Ang II, although unable to provoke sustained hypertension, nonetheless magnifies the pressor response to otherwise trivial stimuli. We speculate that even modestly elevated Ang II levels may contribute to hypertensive complications because such levels promote the punctuation of an apparent normotensive state by episodic hypertension occasioned by seemingly innocuous stimuli. (Hypertension. 2003;42[part 2]:1111-1116.)

Key Words: angiotensin II, sustained hypertension, experimental, blood pressure, vasculature

Chronically infusing a subpressor dose of angiotensin II (SP-Ang II) increases blood pressure after several days.1,2 This hypertensive response, known as the slow pressor response to Ang II, has been shown by many laboratories including our own.3 Most have studied this phenomenon using tail cuff plethysmography (TCP) to measure blood pressure in the conscious animals.1,2,3 Although this technique is very valuable, it does have certain limitations. For instance, it does not allow for continuous monitoring of blood pressure. In addition, the procedure is somewhat cumbersome and involves warming the rats, placing them in restraining cages, and then inflating the tail cuff; all of these steps have been shown to induce a stress response in trained normotensive animals, which consequently may alter the blood pressure.4,5 Recently, a radiotelemetric recording system has been developed, which permits long-term continuous monitoring of blood pressure in animals without the stress of heating, handling, or restraining.6,7 Thus, we incorporated this new technology in our laboratory to enhance our blood pressure monitoring during SP-Ang II–induced hypertension (HTN). Using this methodology, we initially attempted to reproduce our previous findings. However, we were surprised to find that 5 ng/kg per minute of Ang II did not induce HTN when measured by telemetry. In fact, even 50 ng/kg per minute of Ang II did not cause HTN, yet the blood pressures in these same rats were increased when measured by the TCP. Because this discrepancy could not be explained by any errors in the telemetry system, we reasoned that the manipulations that the rats undergo during TCP measurement of blood pressure induce enough stress to elevate blood pressure in rats with enhanced pressor responses to stress. Thus, we designed the current study to test the hypothesis that SP-Ang II increases the pressor response to simple stress without causing sustained HTN in rats. We tested this hypothesis by monitoring continuous blood pressure recordings in unrestrained control and SP-Ang II–treated rats, and by measuring their blood pressure during the TCP procedure by both TCP and simultaneous telemetry recordings.

Methods

Animal Preparation

The Institutional Animal Care and Use Committee approved this study. Adult male Sprague-Dawley rats (250 g; Harlan Laboratories, Indianapolis, Ind) were placed in metabolic cages and maintained on a standard diet and water ad libitum. Five to eight days later, we implanted telemetry devices (TA11PA-C40, Data Sciences International) into each
rat as previously described. Briefly, we anesthetized the rat with ketamine (100 mg/kg body weight; Fort Dodge Laboratories) and xylazine (50 mg/kg body weight; Lloyd Laboratories). We then implanted the transmitter body into the peritoneal cavity and introduced the transmitter catheter through the left femoral artery so that the tip was below the renal arteries. We allowed 10 days for recovery, during which time the rats were trained daily for TCP measurements. Animals were then randomized to receive either intravenous SP-Ang II at 50 ng/kg per minute (n=6), pressor doses of Ang II (pressor-Ang II) at 300 ng/kg per minute (n=8), or vehicle (n=7) intravenously via implanted osmotic minipumps (ALZET 2001 2ML2, Alzet). In this second surgery, the animals only required half the dose of anesthesia. The minipump was implanted in a midscapular pocket, and a polyethylene catheter connected to the minipump was implanted into the jugular vein. We allowed 2 days for recovery from the second surgery, and then we began monitoring continuous blood pressure. For all groups, we collected plasma at baseline (before implanting minipumps) and at days 6 and 12 (after implanting minipumps) from conscious animals to determine plasma Ang II levels (Immuno-Assay, Cayman Chemical Co). For this animals were placed in acrylic restraining chambers. We then warmed their tails for 10 minutes, after which the caudal vein was cannulated with a 23G infusion needle, and then blood was extracted through this needle. Note that we only warmed the tails (not the entire animals) to minimize the stress response. We also collected 24-hour urine samples every 3 days to determine urine flow, sodium excretion, and sodium balance.

**Measurement of Blood Pressure**

Systolic blood pressure (SBP) was recorded continuously in the unrestrained rat via telemetry as the reduced mean of a 10-second sampling obtained every 10 minutes (DataQuest ART). The simultaneous TCP/telemetry readings from restrained animals were recorded and stored separately from the continuous SBP data. For the simultaneous measurements, the rats were placed in acrylic restraining chambers and warmed for 10 to 15 minutes. Then, for the TCP procedure, SBP was measured using a photosensitive pulse sensor connected to a transducer and amplifier (Harvard Apparatus). To test whether changes in SBP obtained by TCP were also detected by telemetry, we placed the TCP device in front of a telemetry receiver and recorded the blood pressure measurements in anesthetized rats. These measurements were obtained from conscious animals to determine plasma Ang II levels (Immuno-Assay, Cayman Chemical Co). For this animals were anesthetized with inactin (0.1 mL/kg), and MAP was measured from the cannulated right femoral artery and then compared with simultaneous telemetry readings.

**Statistical Analysis**

Values are reported as mean ± SE. One-way repeated-measures ANOVA was used to compare differences among groups, and the Tukey test was used for pairwise comparison.

**Results**

**SBP in Unrestrained and Restrained Rat**

There were no changes in SBP in the control group, as measured by either continuous telemetry (125 ± 3 versus 128 ± 5 mm Hg) or simultaneous TCP/telemetry (132 ± 18 versus 145 ± 20 mm Hg). However, the SBPs obtained during simultaneous TCP/telemetry were somewhat elevated, suggesting that a small pressor response was elicited by TCP (Figure 1A). SP-Ang II did not increase continuous SBP in the unrestrained animals (124 ± 2 versus 127 ± 1 mm Hg), but SBP was increased by 25 mm Hg when measured via simultaneous TCP/telemetry (Figure 1B) during SP-Ang II. The magnitude of the increase in SBP was the same by both methods during the simultaneous procedure (134 ± 19 to 159 ± 22 mm Hg by TCP, 131 ± 20 to 154 ± 25 mm Hg by telemetry). Pressor-Ang II increased both continuous SBP (130 ± 3 to 174 ± 15) and simultaneous TCP/telemetry (143 ± 20 to 209 ± 32 TCP and 147 ± 22 to 193 ± 31 telemetry; Figure 1C).

The MAP values in the anesthetized rat, as measured via simultaneous telemetry and intra-arterial catheter, were sim-
ilar in each group. However, only the pressor-Ang II group had hypertension (Figure 2).

Plasma Ang II
Infusion of SP-Ang II caused a modest nonsignificant increase in the plasma levels of Ang II by day 6 (Figure 3), which returned to baseline values by the end of the experiment.

Metabolic Data
The Table shows the body weight, urine flow rate, sodium excretion sodium balance during SP-Ang II, and vehicle infusion. There were no changes in any of these parameters in either group for the duration of the experiments.

Discussion
The current study establishes several potentially important findings with respect to blood pressure responses to chronic infusion of SP-Ang II. First, contrary to our expectations, SP-Ang II infusion did not cause sustained HTN (even at 50 ng/kg per minute), when measured using a continuous telemetry technique. Rather, it caused an exaggerated vasopressor response to simple stimuli: in this case, TCP measurement of blood pressure. This exaggerated response likely accounts for the HTN that we previously reported in rats receiving 5 ng/kg per minute of Ang II intravenously.3

Chronic infusion of SP-Ang II is commonly used to produce experimental HTN.1,2,3 It is a widely used model because it has many features that are thought to be similar to those seen in human essential HTN. We previously reported that chronically infusing 5 ng/kg per minute of Ang II intravenously slowly increases SBP by ≈20 mm Hg as measured by TCP.3 Because of the limitations of measuring SBP, we recently began monitoring blood pressure continuously in the conscious unrestrained rat via a telemetry system. However, much to our surprise, we could not detect an increase in blood pressure using this telemetry system. We first questioned whether this was due to a lack of sensitivity or inaccurate blood pressure measurements obtained with the telemetry method. However, this seemed unlikely because pressure measurements obtained before implanting and after recovering the telemetry transmitters (on completion of the protocols) were quite accurate. Furthermore, we measured intra-arterial blood pressures simultaneously via the telemetry transmitters and a separate intra-arterial catheter connected to a pressure transducer in the anesthetized animals at the completion of the experimental protocols. We found an excellent correlation between the blood pressure measurements obtained with the 2 techniques. These results suggested that the lack of HTN could not be explained by inaccuracies in the blood pressure measurement technique. We also excluded the possibility that there was a lack of delivery of Ang II via the osmotic minipump, and thus we next performed experiments in which sustained HTN should absolutely be present; that is, we infused pressor doses of Ang II intravenously into the rats. Indeed, sustained HTN was easily detected in this protocol. However, interestingly, the blood pressure levels tended to be lower than what is usually reported at these doses of Ang II. Because most studies (including our previous ones) used TCP to monitor blood pressure in the awake rats, we tested whether the TCP measurements may be the reason for the higher values.

The 2 mechanisms by which TCP can potentially give elevated blood pressure readings are (1) via artifact, that is giving a falsely elevated value, or (2) by inducing a pressor response due to the procedure. We considered the first possibility unlikely because it does not explain how control animals have normal blood pressures. In contrast, pressor responses to TCP have been reported in mice and rats.4,9 Thus, we tested the possibility that TCP is causing a pressor response to TCP measurement of blood pressure. This exaggerated response likely accounts for the HTN that we previously reported in rats receiving 5 ng/kg per minute of Ang II intravenously.3
response that is enhanced by SP-Ang II. To do this, we repeated the previous protocol, except that we monitored blood pressure via continuous telemetry and TCP. In addition, we minimized the pressor responses that are usually seen during TCP in untrained animals by submitting them to a prolonged initial training period and then continuing to measure SBP every 2 days throughout the 2 weeks of the experiment, thereby improving the conditioning of our animals. Finally, we ensured that the TCP SBP measurements were comparable to intra-arterial SBP readings by measuring the blood pressure during the TCP maneuvers with simultaneous telemetry. We found that TCP tended to induce a small pressor response in control rats (by 16±7 and 22±10 on days 6 and 12, respectively), similar to what others have reported.4,10 This trend, however, did not reach statistical significance in our study, likely because the increases in SBP were not only less pronounced, but also less consistent than in the Ang II-treated rats.

As mentioned above, this pressor response was significantly greater in the rats receiving SP-Ang II than in controls. This increase in blood pressure was detected by both TCP and simultaneous telemetry. Thus, the methods used in this study allowed us to observe how these animals were reacting to the simple stress of TCP. Specifically, we obtained blood pressure data continuously up to the point at which the animals were removed from the metabolic cages and placed into the restrainers. This restraining/warming process took between 10 and 15 minutes, and then we immediately began collecting the TCP/telemetry data. When we examined the continuous data to the point where we removed the animal from the metabolic cage, there was no change in blood pressure in either the control or SP-Ang II rats. However, when we examined the telemetry data obtained during the period beginning 1 minute before, up through 1 minute after the 5 TCP measurements, the SBP was increased (whether measured by telemetry or TCP). Despite the enhanced pressor response induced by SP-Ang II, the blood pressure uniformly normalized upon return to their cages where they were allowed to roam freely (usually within 30 minutes).

Restraining and warming of the animals for TCP have been shown to induce a stress response in trained normotensive animals, and in fact, restraining elicited a pressor and tachycardic response in normotensive mice4 when BP was monitored by telemetry. Although mice are more sensitive to stress than rats, this response was also shown in SHR rats, suggesting that this strain of rats may have an enhanced pressor response. Interestingly, SHR have high Ang II levels (despite normal plasma renin activity), and the effect of ACE inhibitors in reducing BP is less pronounced when measured by telemetry than with TCP. This may suggest that the enhanced pressor response in SHR may be, in part, mediated by Ang II. Further support for this notion is also provided by the observation that Sprague Dawley rats maintained on a low sodium diet (thus having an activated endogenous renin-angiotensin system) also have a significantly enhanced pressor response to thermal stimuli. Thus, these studies, when taken together with our current one, provide strong evidence that Ang II exaggerates the pressor response to simple stimuli.

We regard our findings as relevant and timely given the wide use of Ang II-induced slow pressor response.13 However, we wish to point out certain considerations and caveats. First, the duration of the current study was only 2 weeks. Therefore, it is quite possible that SP-Ang II may induce sustained hypertension if it is infused for longer periods of time. Second, many studies infuse SP-Ang II subcutaneously rather than intravenously, making it difficult to compare responses to the various doses. Thus, depending on the dose and/or route of administration, SP-Ang II may induce sustained hypertension or enhanced pressor responses. Third, many investigators concomitantly treat their animals with ACE inhibitors, which may sensitize the animals to SP-Ang II effects. Finally, some studies detect sustained hypertension in response to SP-Ang II in chronically catheterized animals. The reason for this apparent discrepancy is unclear but may be due to differences in dose/delivery of Ang II, to the lack of complete freedom of motion caused by the chronic catheters, or to chronic inflammation (with enhanced tumor necrosis factor α) that may potentiate the effect of Ang II. These possibilities clearly need further study.

Perspectives
We found that, although a 2-week infusion of SP-Ang II did not trigger sustained hypertension, remarkably, it magnified the pressor response to otherwise trivial stimuli (eg, TCP). This raises the possibility that diverse factors that usually do not induce hypertension (eg, high salt diet) may interact with SP-Ang II under certain conditions and together bring about a hypertensive response. Thus, we speculate that even modestly elevated Ang II levels may contribute to hypertensive complications because such levels may interact with seemingly innocuous stimuli to promote episodic or perhaps even sustained hypertension.

References
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