

**Aline Nardoni Gonçalves Braga, Marisa Da Silva Lemos, José Roberto Da Silva, Walkiria Ramos Peliky Fontes and Robson Augusto Souza Dos Santos**  
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# Effects of angiotensins on day-night fluctuations and stress-induced changes in blood pressure

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**Braga, Aline Nardoni Gonçalves, Marisa Da Silva Lemos, José Roberto Da Silva, Walkíria Ramos Peliky Fontes, and Robson Augusto Souza Dos Santos.** Effects of angiotensins on day-night fluctuations and stress-induced changes in blood pressure. *Am J Physiol Regulatory Integrative Comp Physiol* 282: R1663–R1671, 2002. First published March 7, 2002; 10.1152/ajpregu.00583.2001.—In this study we evaluated by telemetry the effects of ANG II and ANG-(1–7) infusion on the circadian rhythms of blood pressure (BP) and heart rate (HR) and on the cardiovascular adjustment resulting from restraint stress in rats. ANG II or ANG-(1–7) or vehicle were infused subcutaneously for 7 days. Restraint stress was carried out before, during, and after infusion at 7-day intervals. Parallel with an increase in MAP, ANG II infusion produced an inversion of MAP circadian rhythm with a significant MAP acrophase inversion. It also produced bradycardia during the first 3 days of infusion. Thereafter, HR progressively increased, reaching values similar to or above those of the control period at the end of the infusion period. HR circadian variation was not changed by ANG II infusion. Strikingly, ANG II significantly attenuated the increase in MAP induced by restraint stress without altering the HR response. ANG-(1–7) infusion produced a slight but significant decrease in MAP restricted to the daytime period. No significant changes in the MAP acrophase were observed. In addition, ANG-(1–7) infusion produced a small but significant sustained bradycardia. ANG-(1–7) did not change cardiovascular responses to restraint stress. These data indicate that ANG II can influence the activity of brain areas involved in the determination of stress-induced or circadian-dependent variations of blood pressure without changing HR fluctuations. A significant modulatory influence of ANG-(1–7) on basal MAP and HR is also suggested.

angiotensin II; angiotensin-(1–7); circadian rhythm; restraint stress; telemetry; hypertension

A VARIETY OF PARAMETERS of the cardiovascular system such as blood pressure (BP), heart rate (HR), cardiac output, and stroke volume change rhythmically according to a 24-h cycle, being higher in the active phase than in the resting phase (28, 36). Several cardiovascular events also show 24-h variations, including myo-

cardial ischemia (46), myocardial infarction (39), sudden cardiac death (38), and ischemic stroke (20, 35). It was also reported that end-organ damage occurs with a higher frequency in nondipper hypertensive patients whose BP remains elevated throughout the night (34, 41, 45). In the search for a way to prevent and treat these events with new chronotherapeutic approaches (29, 37), it is important to clarify the mechanisms of such 24-h variations. It has been shown that the rhythm of BP and HR are controlled by an endogenous circadian oscillating system (65) in which the suprachiasmatic nucleus (SCN) plays an important role in rats (24, 48, 49, 57, 62). It is unknown, however, how the circadian information from the SCN is modulated/processed to regulate the 24-h rhythm of BP and HR.

The circadian pattern of arterial pressure is influenced by hormonal factors such as the hypothalamic-pituitary-adrenal and hypothalamic-pituitary-thyroid axes, the renin-angiotensin-aldosterone system, opioids, and various vasoactive peptides (57). Because the renin-angiotensin system (RAS) is one of the major humoral mechanisms controlling BP, it is important to clarify the relation between this system and the circadian rhythm of cardiovascular parameters and how this relation is involved in the mechanisms regulating BP. It was observed by telemetry that an inverse circadian BP pattern occurs in TGR(mREN2)27 transgenic rats (31), which present an overactivity of tissue RAS (19). In these rats, BP values were at maximum during the day around noon, when the rats are in their resting phase. In contrast, in normotensive and spontaneously hypertensive rats, the 24-h BP and HR profiles show peak values during the active phase at night, between midnight and 3:00 AM (26, 31, 60). These data suggest that a direct effect of the RAS may be involved in the disturbed circadian rhythmicity in TGR(mREN2)27 rats.

There is increasing evidence that in addition to ANG II, other smaller angiotensin peptides, including ANG III, ANG IV, and ANG-(1–7) can mediate biological actions of the RAS (50, 51). It was also suggested that

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ANG-(1-7) counteracts most of the cardiovascular actions of ANG II, including vasoconstriction and proliferation (51, 58). In the brain, ANG-(1-7) facilitates baroreflex, whereas ANG II decreases it (2, 9, 50). Furthermore, at the rostral ventrolateral medulla (RVLM), a critical region for determining peripheral sympathetic activity (14), under basal conditions endogenous ANG-(1-7) appears to have mainly an excitatory influence (16, 17, 54), whereas an inhibitory AT<sub>1</sub>-receptor-mediated effect has been suggested for ANG II (16). However, it is unknown how these peptides influence the circadian rhythm of cardiovascular parameters. Thus the aim of the present study was to evaluate the effect of ANG II and ANG-(1-7) infusion on the circadian rhythms of BP and HR. To evaluate whether the changes in the baseline values of MAP and HR were also associated and/or due to changes in autonomic nervous system reactivity, the cardiovascular responses to restraint stress were also determined.

## MATERIALS AND METHODS

**Experimental animals.** Wistar male rats weighing 250 to 310 g were used. All rats were obtained from Cebio-Centro de Bioterismo do Instituto de Ciências Biológicas-Universidade Federal de Minas Gerais. Free access was allowed to standard diet (Nuvilab CR1-Nuvital Nutrientes) and tap water was supplied ad libitum. The rats were housed in separated cages under controlled conditions of temperature (25°C) and a 12:12-h light/dark cycle (light: 6:00 AM to 6:00 PM; dark: 6:00 AM to 6:00 PM). Before the experiments were started, the animals underwent a 12-day acclimatization period in an isolated telemetry room. All experimental protocols were performed in accordance with the guidelines for the human use of laboratory animals of our institute and approved by local authorities.

**Radiotelemetry monitoring of BP and HR.** A telemetry system (Data Sciences International, MN) was used for measuring systolic, diastolic, and MAP and HR and motor activity. This monitoring system consists of a radiofrequency transducer model TA11-PA C40, a receiver, a matrix, and an IBM-compatible personal computer with accompanying software (Dataquest A.R.T., Gold 2.0) to store and analyze the data (6). Under tribromoethanol anesthesia 2.5% (1 ml/100 g body wt), the catheter-transducer was implanted into the abdominal aorta just above the bifurcation of the iliac arteries, and the sensor was fixed to the abdominal wall. Before the experiments were started, the rats were housed in individual cages for 10–12 days until the telemetry tracings indicated reestablishment of 24-h oscillations of BP and HR. Data were sampled every 10 min for 10 s/24 h.

**Restraint stress experiment.** Each stress experiment session was divided in three periods: control (1 h), stress (30 min), and recovery (2–3 h). During the restraint stress the rats were placed in an acrylic transparent triangular container over the receiver panel, and cardiovascular parameters were registered continuously (1 value every 10 s).

**Experimental protocol.** The experimental protocol was initiated with the first restraint stress. After a 7-day interval, a 7-day infusion of ANG II (6 µg/h, *n* = 6) or ANG-(1-7) (6 µg/h, *n* = 5) or vehicle [0.9% NaCl, 1 µl/h (*n* = 4)] was started using osmotic minipumps (ALZET, model 2001) implanted subcutaneously in the dorsal region under tribromoethanol anesthesia (2.5%, 1 ml/100 g body wt). On the 7th day of infusion, the second restraint stress was performed. After a

7-day interval (recovery period), a third restraint stress was performed. All of the stresses were carried out at the same time, 10:00 AM. ANG II and ANG-(1-7) were purchased from Bachem (Torrance, CA).

**Statistical analysis.** Linear data are presented as means ± SE. Circular data (acrophases) are presented as the mean vector (in hours) and 95% confidence interval. The MAP and HR circadian variation were calculated from the night mean values (6:00 PM–5:55 AM) compared with the day mean values (6:00 AM–5:55 PM). For statistical analysis, 72 values for every 12 h (1 value at each 10 min) for each rat were computed. Comparisons between day and night values within the same group were made by unpaired Student's *t*-test (Prism 3.0, Graphpad Software, 1999). Comparisons between the control period and the experimental and recovery periods were made by one-way ANOVA, followed by the Dunnett's test. For this, the control values were obtained by averaging all values obtained in the last 3 days of the preinfusion period. The MAP and HR acrophase, which is the time of the day or night of the peak of their circadian rhythm expressed in hours or in degrees (where 360° corresponds to a 24-h full cycle), were obtained by rhythmic analysis (DQ-FIT software) (67, 69) of all data points collected in the last 3 days of each experimental period (control, infusion, and recovery). Individual acrophases, derived from the DQ-FIT analysis, were averaged and compared using a circular statistics software (Software Oriana, version 1.06, Kovach Computing Services, 1994). The comparisons between the circular mean of the acrophases were performed using the Watson *f*-test (63).

The MAP and HR data of the stress experiments were analyzed by paired Student's *t*-test. The MAP and HR variability (evaluated by the SD of the means of MAP and HR values) was analyzed by one-way ANOVA, followed by the Dunnett's test. A value of *P* < 0.05 was considered statistically significant.

## RESULTS

**Effects of ANG II on the circadian variation and variability of MAP and HR.** As shown in Fig. 1A, in the control period MAP was significantly higher at night (average of the 3 nights before infusion: 99 ± 0.3 mmHg) compared with the daytime values (92 ± 0.2 mmHg). ANG II infusion significantly increased MAP during the day compared with the night period starting within 3 days of infusion, inverting the MAP circadian rhythm (day 3: 125 ± 1.0 mmHg during the day and 120 ± 1.0 mmHg during the night). Accordingly, there was an inversion in the MAP acrophase (control period: 12:53 AM; 95% confidence interval 9:57 PM–3:48 AM vs. infusion period: 2:28 PM; 95% confidence interval 11:32 AM–5:25 PM). These changes had no relationship with alterations in locomotor activity (data not shown). During ANG II infusion there was also an increase in the arterial BP variability estimated by the SD of MAP. As shown in Table 1 in ANG II-infused rats, the MAP SD increased from 8.9 ± 0.5 to 22.3 ± 1.2 mmHg during the day and from 8.7 ± 0.5 to 20.3 ± 1.4 mmHg during the night. After the end of the ANG II infusion there was a progressive decrease in MAP, which reached values similar to those of the control period within 4–5 days of the recovery period (Fig. 1A). However, the MAP variability remained slightly elevated compared with the control period, even during

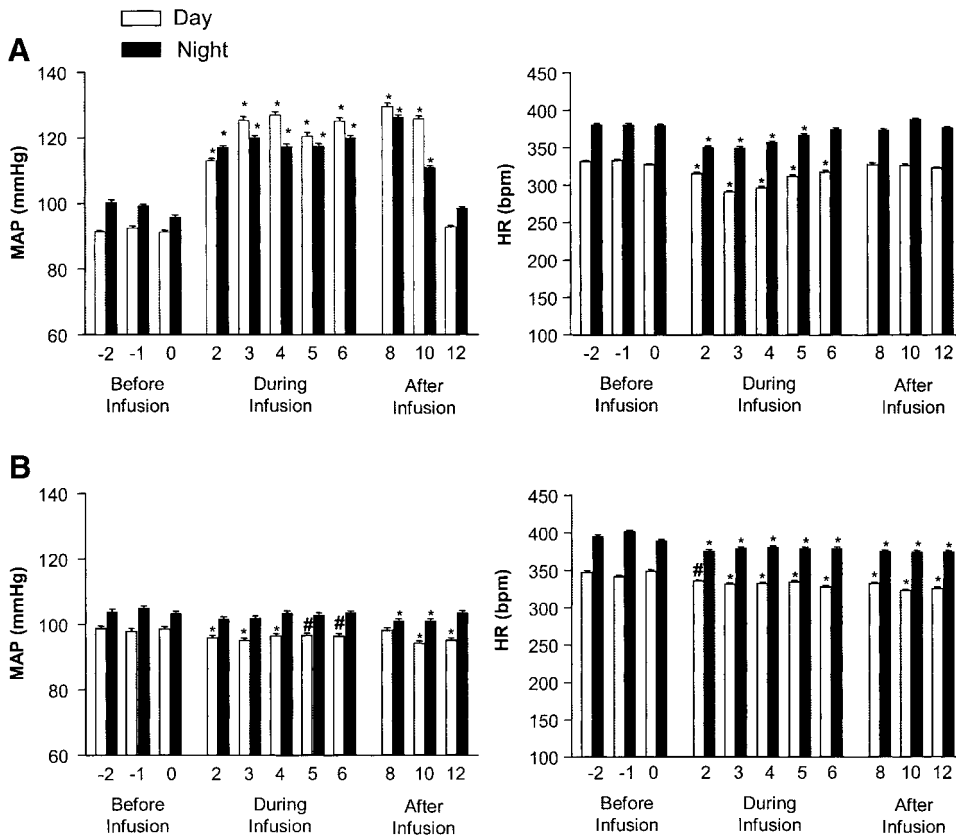


Fig. 1. Effect of chronic infusion of angiotensin peptides on the circadian variation of mean arterial pressure (MAP) and heart rate (HR). A: averaged values of MAP and HR during daytime and nighttime before, during, and after ANG II infusion (6  $\mu\text{g/h}$ ). B: averaged values of MAP and HR during daytime and nighttime before, during, and after ANG-(1-7) infusion (6  $\mu\text{g/h}$ ). \* $P < 0.001$  and # $P < 0.05$  compared with the average of the 3 last days of the period before infusion (ANOVA followed by the Dunnett's test). See METHODS for details. bpm, Beats/min.

the last 3 days of the recovery period (Table 1). Parallel to the increase in MAP there was a significant decrease in HR that was more pronounced in the first 3 days (Fig. 1A). Thereafter, HR values progressively increased, reaching values similar to or above those of the control period at the end of the infusion (Fig. 1A, see also Fig. 2). On the other hand, the circadian variation of HR was not significantly affected by ANG II infusion [average of the 3 last days of the control period: day,  $331 \pm 1.0$  beats/min and night,  $380 \pm 1.7$  beats/min, vs. infusion period (day 3): day,  $290 \pm 2.1$  beats/min, and night  $349 \pm 2.3$  beats/min; acrophase: 11:46 PM; 95% confidence interval 10:34 PM–12:57 AM before infusion vs. 12:22 AM; 95% confidence interval 11:36 PM–1:08 AM, infusion]. Similar to that observed for MAP, ANG II infusion produced an in-

crease in the HR variability (Table 1). Figure 2 shows a recording of BP, HR, and locomotor activity before, during, and after ANG II infusion.

In the group that received vehicle infusion, there were no significant changes in MAP or HR values (Table 2).

*Effects of ANG-(1-7) on the circadian variation and variability of MAP and HR.* As shown in Fig. 1B, ANG-(1-7) infusion produced a slight but significant decrease in MAP. Strikingly, this change was significant only during the resting period (average of 3 last days of the control period:  $99 \pm 0.5$  mmHg vs. day 3, infusion period:  $95 \pm 0.7$  mmHg). ANG-(1-7) infusion did not alter the MAP circadian variation (acrophase in the control period: 1:50 AM; 95% confidence interval 12:03 AM–3:37 AM vs. acrophase during infusion

Table 1. Standard deviation of MAP and HR values in rats infused with ANG II, ANG-(1-7), or vehicle

|               | ANG II<br>(6 $\mu\text{g/h}$ , n = 6) |                  |                        | ANG-(1-7)<br>(6 $\mu\text{g/h}$ , n = 5) |                 |                | Saline<br>(1 $\mu\text{l/h}$ , n = 4) |                 |                |
|---------------|---------------------------------------|------------------|------------------------|--|-----------------|----------------|---------------------------------------|-----------------|----------------|
|               | Before infusion                       | During infusion  | After infusion         | Before infusion                          | During infusion | After infusion | Before infusion                       | During infusion | After infusion |
| MAP, mmHg     |                                       |                  |                        |  |                 |                |                                       |                 |                |
| Day           | $8.9 \pm 0.5$                         | $22.3 \pm 1.2^*$ | $20.2 \pm 4.4^*$       | $11.5 \pm 0.8$                           | $9.8 \pm 0.3^*$ | $10.9 \pm 0.5$ | $10.8 \pm 0.5$                        | $12.8 \pm 0.4$  | $13.8 \pm 0.2$ |
| Night         | $8.7 \pm 0.5$                         | $20.3 \pm 1.4^*$ | $15.9 \pm 3.3^*$       | $12.6 \pm 0.3$                           | $13.3 \pm 0.3$  | $14.3 \pm 0.5$ | $10.2 \pm 0.1$                        | $12.6 \pm 0.4$  | $13.0 \pm 0.3$ |
| HR, beats/min |                                       |                  |                        |  |                 |                |                                       |                 |                |
| Day           | $37.6 \pm 0.7$                        | $59.2 \pm 2.6^*$ | $53.8 \pm 8.3^*$       | $38.4 \pm 1.5$                           | $36.6 \pm 0.8$  | $36.6 \pm 2.9$ | $36.5 \pm 3.5$                        | $36.5 \pm 0.8$  | $35.7 \pm 0.8$ |
| Night         | $48.3 \pm 0.5$                        | $58.2 \pm 0.9^*$ | $50.8 \pm 4.3^\dagger$ | $44.6 \pm 0.6$                           | $44.2 \pm 0.7$  | $42.7 \pm 0.2$ | $37.2 \pm 1.6$                        | $37.1 \pm 0.7$  | $37.1 \pm 1.4$ |

The numbers represent the means  $\pm$  SE of the SD of mean arterial pressure (MAP) and heart rate (HR) values collected in the last 3 days of each 7-day period (before, during, and after infusion). \* $P < 0.05$  compared with control period;  $\dagger P < 0.05$  after infusion vs. infusion.

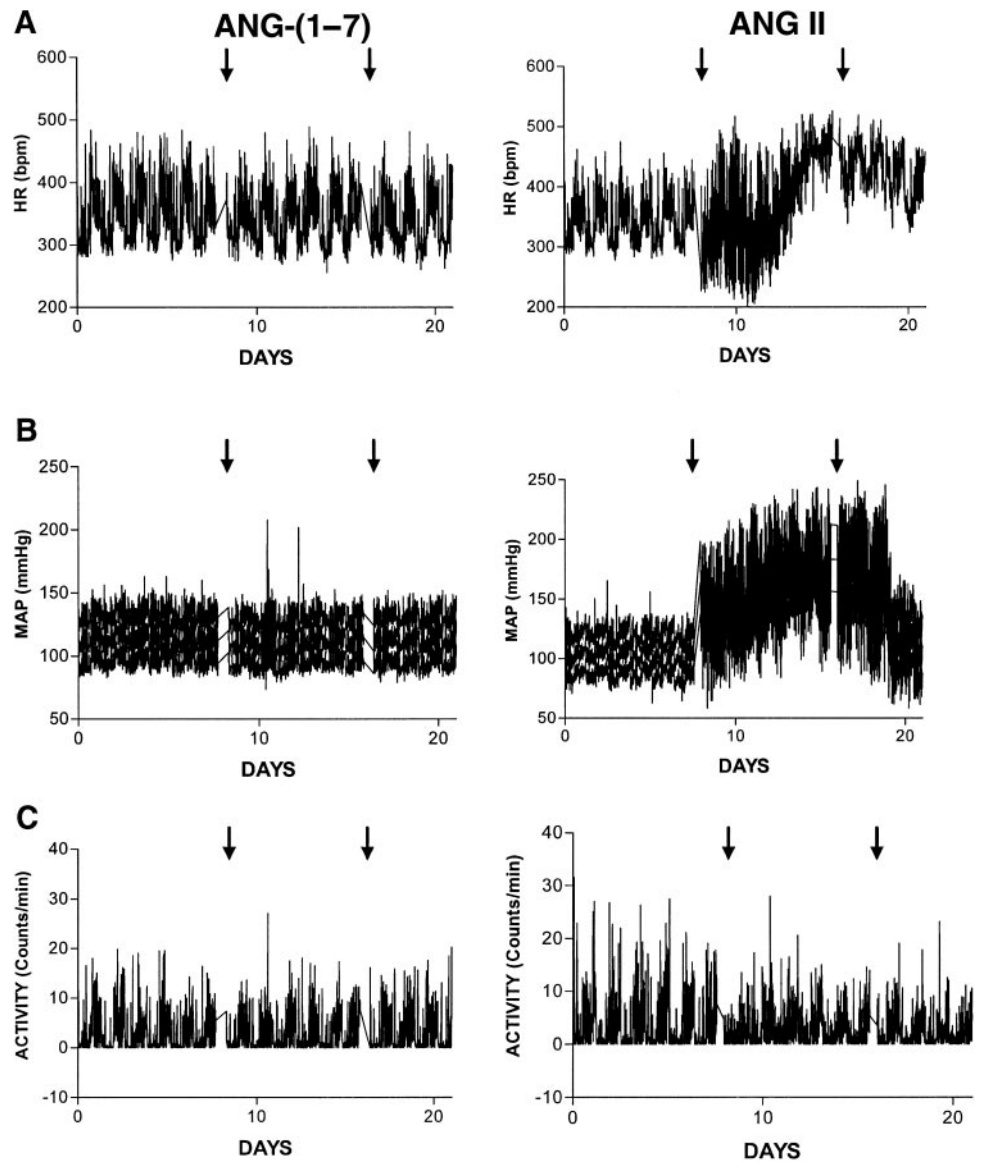


Fig. 2. Representative recordings of the effects of the infusion of ANG-(1-7) (6  $\mu$ g/h) and ANG II (6  $\mu$ g/h) on heart rate (A, beats/min), blood pressure (B, mmHg), and activity (C, counts/min). Arrows indicate the beginning and end of infusion. bpm, Beats/min.

12:27 AM; 95% confidence interval 11:43 PM–1:11 AM). As observed for ANG II, during the ANG-(1-7) infusion there was a small but significant bradycardia that was still present in the recovery period (average of the 3 last days of the control period: day,  $343 \pm 1.7$

beats/min and night,  $395 \pm 1.8$  beats/min, vs. day 3 of infusion period: day,  $331 \pm 1.9$  beats/min, and night  $379 \pm 2.1$  beats/min). The HR acrophase did not change with ANG-(1-7) infusion: control, 12:17 AM; 95% confidence interval 11:52 PM–12:43 AM and infu-

Table 2. MAP and HR before, during, and after subcutaneous infusion of saline

|               | Before Infusion | During Infusion |               |               |               |               | After Infusion |               |               |
|---------------|-----------------|-----------------|---------------|---------------|---------------|---------------|----------------|---------------|---------------|
|               | CT              | Day 2           | Day 3         | Day 4         | Day 5         | Day 6         | Day 2          | Day 4         | Day 6         |
| MAP, mmHg     |                 |                 |               |               |               |               |                |               |               |
| Day           | $90 \pm 0.5$    | $90 \pm 0.6$    | $90 \pm 0.7$  | $92 \pm 0.7$  | $91 \pm 0.7$  | $91 \pm 0.8$  | $91 \pm 0.7$   | $89 \pm 0.7$  | $88 \pm 0.7$  |
| Night         | $94 \pm 0.4$    | $94 \pm 0.6$    | $93 \pm 0.7$  | $94 \pm 0.6$  | $94 \pm 0.6$  | $94 \pm 0.6$  | $94 \pm 0.6$   | $93 \pm 0.7$  | $93 \pm 0.6$  |
| HR, beats/min |                 |                 |               |               |               |               |                |               |               |
| Day           | $336 \pm 1.4$   | $340 \pm 1.8$   | $343 \pm 1.9$ | $344 \pm 2.1$ | $343 \pm 1.9$ | $339 \pm 2.3$ | $330 \pm 2.0$  | $332 \pm 1.9$ | $331 \pm 1.8$ |
| Night         | $370 \pm 1.4$   | $374 \pm 1.9$   | $372 \pm 2.0$ | $372 \pm 1.9$ | $367 \pm 1.8$ | $371 \pm 2.1$ | $363 \pm 2.1$  | $363 \pm 1.9$ | $356 \pm 1.9$ |

The before infusion values (CT) are the average of the last 3 days of the preinfusion period (see METHODS for details)  $\pm$  SE. All night values are significantly higher than day values ( $P < 0.001$ , unpaired  $t$ -test). The MAP and HR values were collected in 4 rats (as described in METHODS).

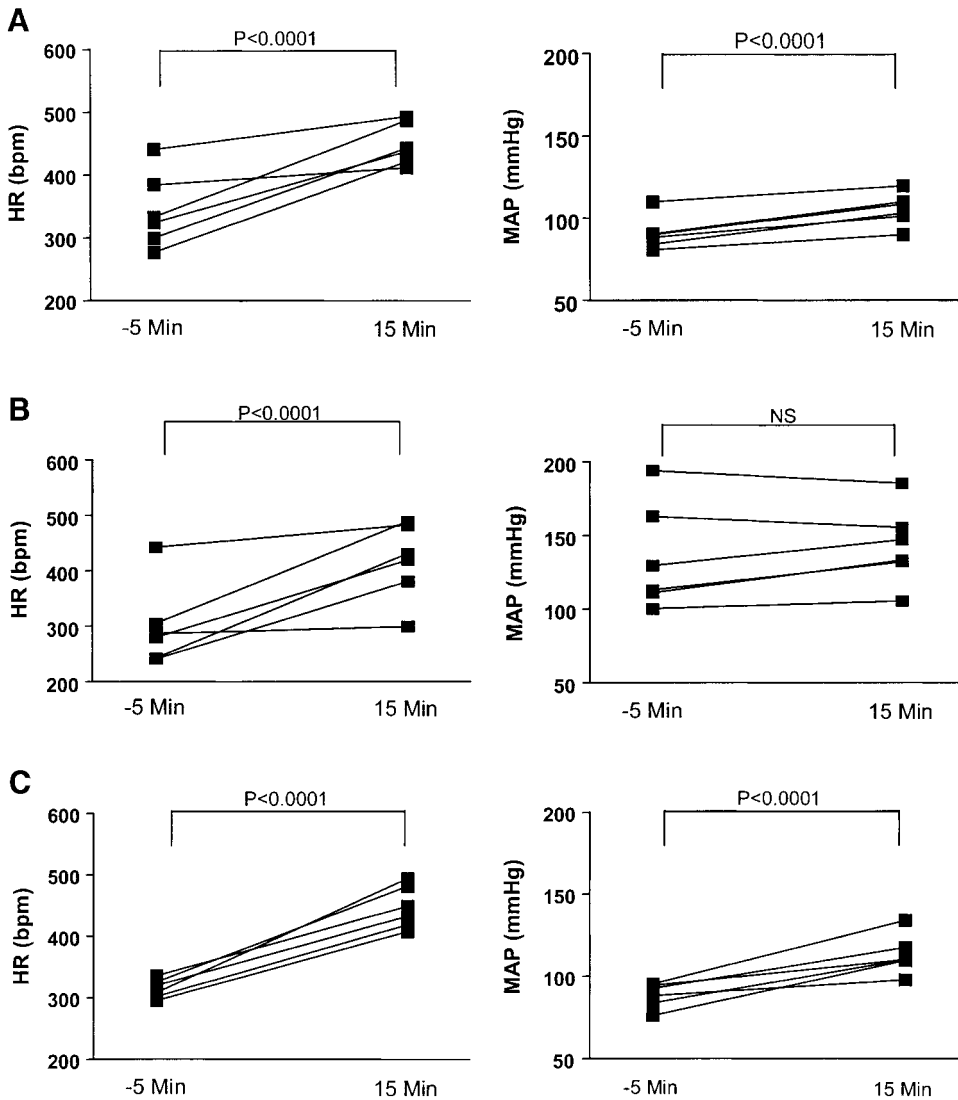


Fig. 3. Effect of ANG II infusion on the cardiovascular responses to restraint stress. Data show the individual values of HR and MAP before (-5 min) and 15 min after the beginning of the restraint stress. A: before infusion; B: day 7 of ANG II infusion (6  $\mu$ g/h); and C: at the end of the recovery period. NS, not significant.

sion, 12:19 AM; 95% confidence interval 11:52 PM–12:46 AM. Conversely to that observed with ANG II infusion, ANG-(1–7) slightly decreased MAP variability during the resting phase (Table 1). ANG-(1–7) infusion did not change nocturnal MAP variability or HR variability (Table 1). Figure 2 shows a typical recording of BP, HR, and locomotor activity before, during, and after ANG-(1–7) infusion.

*Effects of angiotensin peptides on cardiovascular responses to restraint stress.* As shown in Fig. 3 and Table 3, restraint stress produced a significant increase in MAP and HR. Unexpectedly, ANG II infusion significantly attenuated rather than facilitated the increase in MAP produced by restraint stress (before stress:  $135 \pm 14.8$  mmHg vs. stress:  $143 \pm 10.9$  mmHg;  $P > 0.05$ , paired  $t$ -test,  $n = 6$ ), without changing the HR

Table 3. Cardiovascular changes produced by restraint stress in ANG-(1–7) and vehicle-treated rats

| Group                             |         | Before Infusion |                 | During Infusion |                 | After Infusion |                 |
|-----------------------------------|---------|-----------------|-----------------|-----------------|-----------------|----------------|-----------------|
|                                   |         | MAP, mmHg       | HR, beats/min   | MAP, mmHg       | HR, beats/min   | MAP, mmHg      | HR, beats/min   |
| ANG-(1-7) (6 $\mu$ g/h, $n = 5$ ) | Control | 96 $\pm$ 6.8    | 327 $\pm$ 11.6  | 90 $\pm$ 4.8    | 312 $\pm$ 9.4   | 94 $\pm$ 7.0   | 319 $\pm$ 20.6  |
|                                   | Stress  | 111 $\pm$ 8.2*  | 441 $\pm$ 14.7* | 109 $\pm$ 6.4*  | 420 $\pm$ 10.4* | 108 $\pm$ 7.5* | 412 $\pm$ 12.0* |
| Saline (1 $\mu$ l/h, $n = 4$ )    | Control | 85 $\pm$ 4.2    | 321 $\pm$ 15.7  | 90 $\pm$ 5.6    | 340 $\pm$ 14.7  | 83 $\pm$ 6.8   | 313 $\pm$ 10.8  |
|                                   | Stress  | 104 $\pm$ 5.9*  | 417 $\pm$ 16.5* | 101 $\pm$ 5.7*  | 401 $\pm$ 4.6*  | 100 $\pm$ 6.9* | 389 $\pm$ 8.7*  |

Control and stress values are means  $\pm$  SE ( $n = 5$  and  $4$  for ANG-(1–7) and saline, respectively) of data collected 5 min before and with 15 min of stress. \* $P < 0.05$  compared with control period. The values for MAP in mmHg and HR in beats/min were collected continuously (1 value/10 s) as described in methods.

response (before stress:  $300 \pm 30.3$  beats/min vs. stress:  $417 \pm 28.8$  beats/min;  $P < 0.0001$ , paired *t*-test,  $n = 6$ ) (Fig. 3). At the end of the recovery period when the resting values of MAP and HR returned to the control values, the responses to the restraint stress normalized. ANG-(1–7) or vehicle infusion did not alter the changes in MAP and HR resulting from restraint stress (Table 3).

## DISCUSSION

In this study we found that chronic ANG II infusion in Wistar rats inverted the circadian rhythm of BP. Our results are in accordance with the findings that TGR(mREN2)27 rats, which present an overactive tissue RAS, develop an inverted 24-h rhythm of BP (31, 66). The related observation that ANG II infusion inverts day-night rhythms of BP in Sprague-Dawley but not in TGR(ASrAOGEN) rats (3), which present severe inhibition of the angiotensinogen synthesis exclusively in the brain, indicates that a functional brain RAS is also important for the effects of peripheral administration of ANG II on 24-h BP rhythm. It was recently described that an abolished 24-h circadian rhythm in *c-fos* mRNA expression in the SCN and blunted induction of *c-fos* mRNA after application of light pulses in transgenic hypertensive rats (30). Furthermore, BP reductions in TGR(mREN2)27 rats by angiotensin converting enzyme (ACE) inhibitors (32) and angiotensin AT<sub>1</sub>-receptor antagonists (52) are greater during daytime than during the night, reinforcing the hypothesis that the RAS has important relationships with circadian rhythm mechanisms.

It has been supposed that the recurrent day-night variation in BP could be simply a consequence of the rest-activity cycle (27). Our results and those obtained in TGR(mREN2)27 rats (31) and in aged spontaneously hypertensive rats (40) argue against this view. A more appropriate interpretation for these observations would include, in addition to the rest-activity cycle, the influence of ventrolateral medulla (55) and rostral hypothalamic structures, including the SCN, in modulating BP fluctuations (23). One may argue that the changes in MAP circadian variations produced by ANG II were simply due to the increase in BP. However, spontaneously hypertensive rats presented normal circadian variation of MAP (26, 60). In addition, as shown in Fig. 1, on *day 2* of ANG II infusion the increase in MAP was similar of that observed in the subsequent days. However, there was no inversion of the circadian variation of MAP at this time point. We recently reported the effects of microinjection of liposome-entrapped ANG-(1–7) into the RVLM of rats instrumented for telemetric recording of BP (55). In this condition, ANG-(1–7) produced a sustained increase in MAP (5 days) associated with a more transient attenuation of its circadian variation (3 days), showing again dissociation of the levels of BP and its 24-h fluctuation. Thus it is not conceivable that the alterations in MAP circadian variation produced by ANG II

in our conditions could be attributed simply to its pressor effect.

As observed previously in TGR(mRen2)27 (31), the shift in BP acrophase, produced by chronic infusion of ANG II, was not accompanied by changes in HR. A similar finding was reported in Sprague-Dawley rats (3). These observations indicate that HR and BP circadian variabilities are differentially regulated and that this phenomenon is not strain specific. The neural substrate for this difference remains to be elucidated. The fact that SCN lesions alters HR, BP, and locomotor 24-h variability indicate that other brain regions might be involved in the differential changes in HR and BP observed in our study. However, it cannot be excluded that ANG II could influence HR and BP differentially at the SCN due to its differential effects on neuronal activity in this nucleus (59).

The increase in MAP produced by ANG II infusion was associated with significant bradycardia, which peaked on *day 3* of infusion. Thereafter, HR progressively continued reaching values similar to those of the control period at the end of the infusion. The mechanism of these changes in HR was not investigated in our study. However, a decrease in baroreflex sensitivity induced by ANG II acting at blood-brain barrier-deficient areas, such as the subfornical organ and the area postrema is likely involved in the progressive reversal of the initial bradycardia (2, 44).

Parallel to the increase in MAP, ANG II infusion increased MAP variability, evaluated by its SD. Contrasting with the differential changes in MAP and HR circadian rhythm, the changes in MAP variability were accompanied by an increase in HR variability. These alterations probably also reflect the effect of ANG II on the baroreflex function (2, 9). Indeed, a depressed baroreflex as expected in our experimental conditions is generally associated with an increase in MAP and HR lability (1).

Contrasting with the data obtained for ANG II, chronic infusion of ANG-(1–7) produced a small but significant decrease in MAP associated with a slight decrease in MAP variability. No changes in circadian variation of HR were observed. A small but significant bradycardia was also found. The absence of an effect of peripheral infusion of ANG-(1–7) on MAP circadian variation differs from a recent observation of our laboratory showing a significant effect of liposome-entrapped ANG-(1–7) microinjected into the RVLM on MAP acrophase (55). Taken together, these results indicate that ANG-(1–7) receptors are not present in neuronal elements outside the blood-brain barrier, implicated in BP circadian rhythm modulation. It should be pointed out, however, that chronic increases in circulating ANG-(1–7) can influence baroreflex sensitivity (4), suggesting selective expression/action of ANG-(1–7) receptor in cardiovascular-related neuronal pathways.

Previous studies addressing the cardiovascular effects of peripheral administration of ANG-(1–7) used acute recordings (4, 5), chronic catheter implantation into carotid arteries (64), or plethysmography (56). Us-

ing a more appropriate methodology for detection of small changes in cardiovascular parameters, we were able to unveil a significant effect of ANG-(1-7) on MAP and HR. The small but significant decrease in HR is probably related to a central effect of ANG-(1-7) in brain areas associated with vagal activity modulation (8, 13). Indeed, microinjection of ANG-(1-7) into the vagal-solitary complex induces bradycardia mediated by an increase in vagal tonus (8). Although the area postrema-NTS region is a likely candidate for mediating the bradycardia effect of ANG-(1-7) further studies are necessary to confirm this hypothesis.

The slight but significant decrease in MAP produced by chronic infusion of ANG-(1-7) is in agreement with several previous studies showing a modest direct effect of ANG-(1-7) on vascular tonus (7, 15, 33, 42, 43, 51) and NO release (12, 21, 53). It should be considered that the small hypotensive effect was obtained in undisturbed conditions in presence of fully functional cardiovascular reflexes. The recent observations that the vasodilator effect of ANG-(1-7) is increased by ACE inhibitors (15, 42) open the possibility that the cardiovascular effects of this heptapeptide could be increased in special circumstances such as chronic antihypertensive therapy (15, 22, 51). Strikingly, the hypotensive effect of ANG-(1-7) was present only during the resting period. This may be one of the reasons why other studies using acute recordings during the day (the rat resting phase), and thus in a more stressful condition for the animals, did not describe any changes in BP (4, 56, 64).

Several studies suggested an important participation of the RAS on the physiological response to stressful stimuli (18, 61, 68). Renin secretion and ANG II plasma levels are increased in several types of acute or chronic stress (68). The involvement of the RAS in stress is also suggested by the presence of ANG II receptors and ANG II immunoreactivity in brain areas involved in the neuroendocrine and autonomic responses to stressful stimuli (10, 11, 47). Furthermore, ANG II is well known as a facilitatory influence for the sympathetic activity (2). However, to our knowledge, our study is the first to examine the effect of long-term infusion of ANG II on the cardiovascular responses to a stressful condition. Surprisingly, the MAP increase usually observed in response to the restraint stress was not augmented in the ANG II-treated rats. Actually, the response was significantly decreased by ANG II. One may argue that this attenuation could be due to the prevailing levels of BP. However, in L-NAME-treated rats with similar MAP increases we were unable to show any significant change in the MAP increases during restraint stress (MS Lemos and RAS Santos, unpublished results). Further studies have to address whether RVLM-related pathways participate in the attenuating influence of ANG II in the cardiovascular responses triggered by restraint stress (2, 16). Additionally, the use of different models of stress is necessary to clarify whether the ANG II effects are dependent on neural substrates involved in the cardiovascular responses evoked by the stressful condition.

In summary, the data obtained with chronic infusion of angiotensin peptides indicate a major role for ANG II in the modulation of the circadian and stress-induced fluctuations of MAP but not HR. A significant influence of ANG-(1-7) on the mechanisms determining basal MAP and HR is also suggested.

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