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RESEARCH PAPER

Cardiovascular reactivity after blockade of angiotensin AT_1 receptors in the experimental model of tilting test in conscious rats

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Background and purpose: Studies have shown that the angiotensin II AT_1 receptor antagonist, losartan, accentuates the hypotensive response in the orthostatic stress test (tilt) performed in anaesthetized rats. The same effect was not reported with other AT_1 antagonists. The aim of this study was to re-evaluate the effects of AT_1 receptor blockade on the cardiovascular response to tilt in a model developed for conscious rats.
Experimental approach: Rats ($n=5$–7 per group) were instrumented for infusion of drugs and recording of cardiovascular parameters and, after recovery, placed in a plastic tube positioned over the tilt board. The tilt test was conducted by raising the head side of the tilt board from horizontal position to 75° head up position for 15 min.
Key results: Compared with control group (NaCl 0.9%, 1 ml kg^{-1}), oral treatment with 1 mg kg^{-1} per day of losartan or telmisartan did not alter the blood pressure response during tilt. With the 10 mg kg^{-1} dose, both antagonists altered the blood pressure response during tilt (mean maximum changes -11 ± 3 mm Hg; $P<0.01$). A post-tilt hypotension was observed with both doses in losartan and telmisartan groups (-13 ± 1 and -9 ± 2 mm Hg, respectively; $P<0.01$).
Conclusions and implications: The present results indicate that the effect of losartan on the cardiovascular reactivity to tilt shares a similar profile to that of other AT_1 antagonists. Evidence discussed addresses the importance of using a conscious model for testing the influence of antihypertensive drugs on the cardiovascular reactivity to orthostatic challenges.
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Keywords: tilt test; conscious rats; angiotensin receptors; losartan; orthostatic stress

Abbreviations: Ang II, angiotensin II; AT_1, type 1 angiotensin II receptor

Introduction

Angiotensin II (Ang II) is involved in the control of cardiovascular system exerting peripheral and central effects that are mainly mediated by type 1 angiotensin II (AT_1) receptors. Ang II is also involved in a number of pathophysiological conditions such as hypertension and left ventricular hypertrophy (Ferrario, 2006). Therefore, development of the AT_1 receptor antagonists provided important pharmacological tools for the treatment of high blood pressure.

Previous studies have shown that the AT_1 receptor antagonist, losartan, accentuated the orthostatic hypotensive response in the orthostatic stress model carried out in rats (Ohlstein *et al*., 1992; Hashimoto *et al*., 1999; de Moura *et al*., 2005). The same effect was not observed with other highly selective AT_1 receptor antagonists, suggesting that at least peripherally, the orthostatic hypotensive effect caused by losartan was due to effects other than the antagonism of AT_1 receptors. However, in all these studies, the orthostatic stress test was performed in anaesthetized rats (Ohlstein *et al*., 1992; Hashimoto *et al*., 1999; de Moura *et al*., 2005). Although the use of anaesthesia is sometimes necessary in different experimental approaches, it is also known that anaesthetics alter the central sympathetic output (Shimokawa *et al*., 1998). Consequently, in presence of anaesthesia, the cardiovascular reactivity to orthostatic stress might be different in presence of different drugs and this potential interaction might be particularly critical for those that cross the blood brain barrier, as is the case for AT_1 antagonists (Li *et al*., 1993; Gohlke *et al*., 2001). In addition, anaesthetics by themselves activate the renin–angiotensin system, increasing the circulating levels of Ang II, through a compensatory response to anaesthesia-induced inhibition of the sympathoadrenal system (Mirenda and Grissom, 1991; Colson *et al*., 1999). Therefore, a more physiological assessment of the influence of AT_1 antagonists on the cardiovascular reactivity to orthostatic stress in animals would be achieved in absence of anaesthesia.

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The aim of this study was to re-evaluate the effects of AT_1 receptor blockade on the cardiovascular response to orthostatic stress in a model developed for conscious rats. We found that the effect of losartan on the blood pressure-compensatory response during short-term orthostatic cardiovascular challenge exhibits a profile shared by those of other highly selective AT_1 antagonists. Some of these results have been published in abstract form (Bedette and Fontes, 2005).

Methods

All animal procedures were approved by our local Institutional Animal Welfare Committee (CETEA Protocol 206/2006) and conformed to the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals (NIH Publication 80–23, revised in 1996). All efforts were made to minimize the number of animals and their suffering.

Experiments were performed on male Wistar rats (240–300 g) bred at the animal facilities of the Biological Sciences Institute (CEBIO, UFMG, Belo Horizonte, Minas Gerais, Brazil). One day before the experiment, rats were anaesthetized with tribromoethanol (250 mg kg^{-1}, intraperitoneally) and the adequacy of anaesthesia was verified by the absence of a withdrawal response to nociceptive stimulation of a hind paw. Supplemental doses of anaesthetic were administered if necessary. Polyethylene catheters (PE 10 in PE 50) were then placed into a femoral vein and femoral artery for infusion of drugs and recording of cardiovascular parameters, respectively. Both catheters were filled with saline and exteriorized to the interscapular space. Lidocaine (2%) was administered locally at the end of the surgery. Animals were housed individually and permitted free access to food and water. A period of at least 24 h was allowed for recovery, and 2 h before the experiments, the rats were acclimatized to the recording room. Mean arterial pressure (MAP) and heart rate (HR) were continuously recorded with a transducer connected to a data acquisition system (Power Lab; ADInstruments, Bella Vista, NSW, Australia). The pressure transducer was maintained at the level of the heart so that tilting did not influence blood pressure measurement.

Experimental design

Separate groups of rats were treated for 1 week with losartan (Biolab, São Paulo, Brazil) or telmisartan (Boehringer Ingelheim, São Paulo, Brazil), with oral doses of 1 and 10 mg kg^{-1} per day added in the drinking water. Control rats were given tap water for drinking. The efficacy of oral treatment was evaluated by testing the pressor effect produced by different doses of intravenous injections of Ang II (from 0.01 to 0.5 μg kg^{-1}) after different doses of losartan or telmisartan. Ang II was injected in a volume of 1 ml kg^{-1} and the catheter was flushed with 0.2 ml of saline.

For the control experiments, vehicle (saline 0.9%, 1 ml kg^{-1}, $n=7$) was administered intravenously at the day of experiment and in a separate group of rats. To test the efficacy of the method of tilt test in conscious rats in detecting orthostatic hypotension, an additional group of rats was injected with prazosin plus atenolol (0.1 and 2.5 mg kg^{-1}, intravenous, respectively, $n=5$). Adrenergic blockade was used to validate the method, since interference with sympathetic reactivity is expected to cause orthostatic hypotension (Lamarre-Cliche and Cusson, 2001; Goldstein *et al*., 2002).

At the beginning of the experiment, the animals were kept in home cages for 10 min and basal levels of MAP and HR were recorded. For the rats treated intravenously, an additional period of 10 min was included in order to evaluate the cardiovascular effects produced by the drugs. Then, the rats were placed in front of and allowed to spontaneously walk into the opaque plastic tube (tilt container) fixed over the tilt board. The tilt container was closed and set in horizontal position for 10 min. The tilting test (a test of postural hypotension) was conducted by raising the head side of a tilt board from a horizontal position within approximately 2 s. Rats were subjected to a 75° head-up tilt for 15 min. At the end of the tilting test, rats were returned to the horizontal position (recovery period) for further 15 min. The animals for which data were reported remained in good health throughout the course of drug treatment and before or after surgical procedures, as assessed by appearance, behaviour and maintenance of body weight.

Data analysis

The baseline values of MAP and HR were measured as the average values of these variables over the 10-min period immediately preceding the tilting test. Changes from baseline evoked during tilt or in the post-tilt period were assessed by paired *t*-test. Comparisons between the vehicle-treated group and the drug-treated groups for each time point were performed by two-way analysis of variance (factors drug and time) followed by Bonferroni's *post hoc* test. The inhibition of the pressor effect evoked by different doses of Ang II in the presence of losartan or telmisartan was evaluated by one-way analysis of variance followed by the Newman–Keuls test. All data were analysed by GraphPad Prism Software Inc. (San Diego, CA, USA). A value of $P<0.05$ was considered to indicate statistical significance. All values are presented as mean ± s.e.mean.

Results

Average baseline levels of MAP and HR and levels of MAP and HR after different drug treatments are presented in Figure 1. Only the groups receiving prazosin plus atenolol, and losartan or telmisartan (10 mg kg^{-1}) displayed a significant reduction in the baseline blood pressure levels when compared with control values ($P<0.05$).

Figure 2 shows that in the vehicle-treated group, the transition period from horizontal to the 75° head-up tilt in conscious rats evoked variable and non-significant changes in MAP and HR (mean maximum changes 4 ± 3 mm Hg; −28 ± 17 bpm; $P>0.05$ vs baseline). During tilt, MAP was well maintained and a small but significant increase was observed at the end of the maneuvre (7 ± 2 mm Hg; $P<0.05$

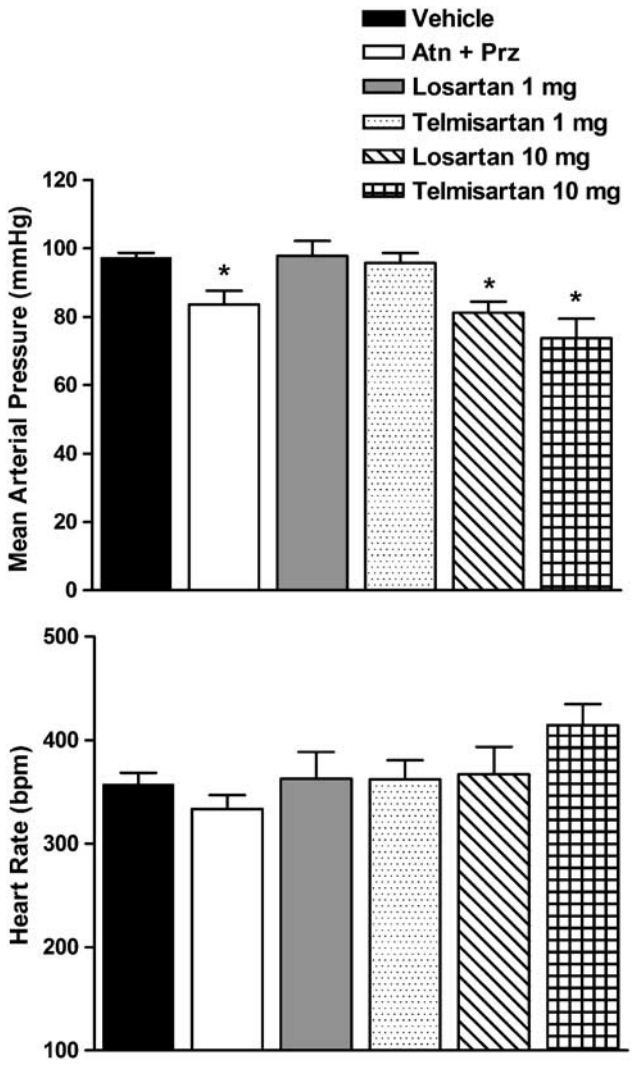

Figure 1 Effects on baseline cardiovascular variables evoked by treatment with atenolol plus prazosin (2.5 and 0.1 mg kg^{-1}, $n=5$), losartan (1 and 10 mg kg^{-1}, $n=6$) or telmisartan (1 and 10 mg kg^{-1}, $n=5$) in conscious normotensive rats. Values represent the mean ± s.e.mean. *$P<0.05$ compared with the vehicle group (0.9% NaCl, 1 ml kg^{-1}; $n=7$). One-way analysis of variance followed by Newman–Keuls test.

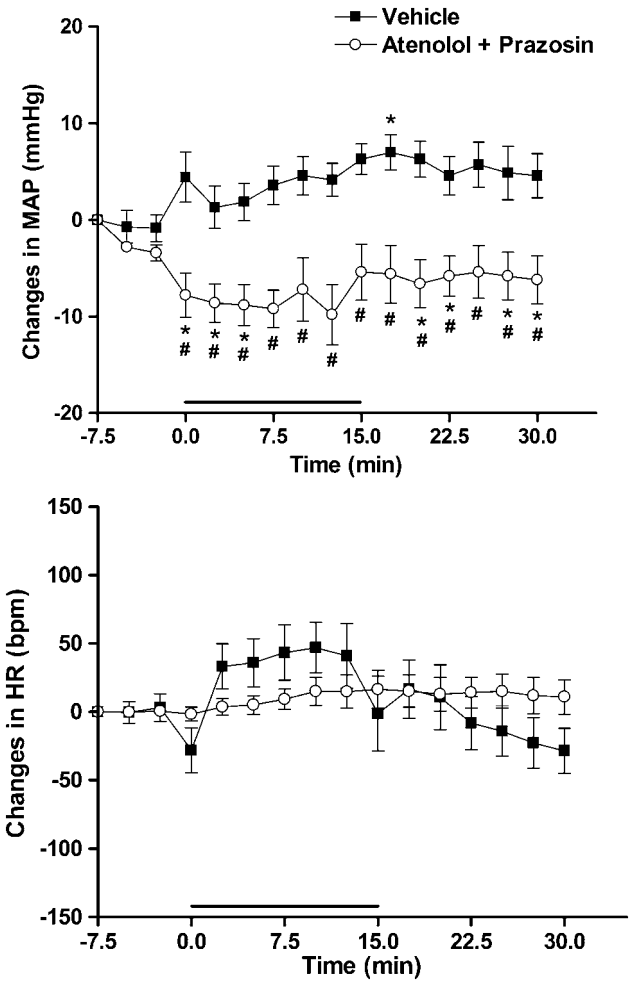

Figure 2 Changes in MAP and HR caused by orthostatic stress (tilt) in conscious rats after intravenous injection of vehicle (0.9% NaCl, 1 ml kg^{-1}; $n=7$) and atenolol plus prazosin (2.5 and 0.1 mg kg^{-1} respectively, $n=5$). Bar indicates the duration of the maneuvre (0–15 min). *$P<0.05$ vs baseline; $^{\#}P<0.01$ vs vehicle group. HR, heart rate; MAP, mean arterial pressure.

vs baseline). This effect was accompanied by variable and non-significant changes in HR, which lasted for the remaining tilting period (mean maximum changes 47 ± 18 bpm). Both cardiovascular variables gradually returned to baseline levels after the maneuvre. As expected, complete adrenoceptor blockade (prazosin plus atenolol, 0.1 and 2.5 mg kg^{-1} intravenous, respectively) induced an orthostatic hypotensive effect (mean maximum changes −9 ± 2 mm Hg; $P<0.03$ vs baseline and $P<0.01$ vs vehicle). This treatment also attenuated the HR variability evoked by tilting observed in the vehicle group, but no significant statistical changes were observed (Figure 2).

Compared with the vehicle group ($n=7$), dosing at 1 or 10 mg kg^{-1} of both losartan and telmisartan inhibited the pressor response to different doses of exogenous Ang II (Figure 3). In this protocol, when a difference between antagonists was found, telmisartan was always more potent than losartan in blunting the pressor effects of Ang II. With the highest dose of AT_1 antagonists, the pressor response obtained with 0.1 μg kg^{-1} of Ang II was reduced by 74% in presence of losartan and by 94% in the presence of telmisartan ($P<0.01$; Figure 3). When compared with control group, oral treatment with the lower dose of losartan or telmisartan (1 mg kg^{-1}) did not induce significant changes in the cardiovascular parameters during the tilt maneuvre (Figure 4). However, a post-tilt hypotension was observed in both the groups (mean maximum changes, −8 ± 1 mm Hg losartan and −8 ± 2 telmisartan, $P<0.01$; Figure 4). When compared with the vehicle group, the 10 mg kg^{-1} dose of both antagonists altered the blood pressure response during tilt (mean max changes −11 ± 3 mm Hg; $P<0.01$; Figure 5). Regarding this specific dose, it is important to point out that no significant difference was found between antagonists during tilt or in the post-tilt period ($P=0.26$). Neither

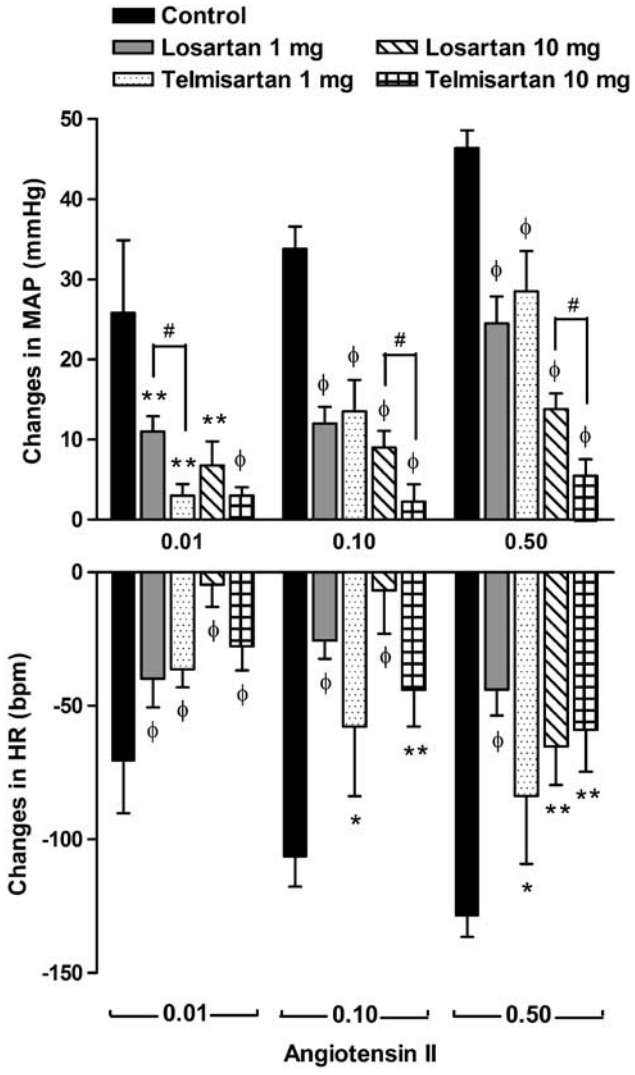

Figure 3 Effect of oral treatment with losartan (1 and 10 mg kg^{-1}, $n=6$) and telmisartan (1 and 10 mg kg^{-1}, $n=5$) on the pressor response to Ang II (0.01, 0.1 and 0.5 μg kg^{-1}, intravenous) in conscious normotensive rats. Values represent the mean ± s.e.mean. *$P<0.05$ compared with control; **$P<0.01$ compared with control; $^{\Phi}P<0.001$ compared with control; $^{\#}P<0.05$ telmisartan vs losartan. Ang II, angiotensin II.

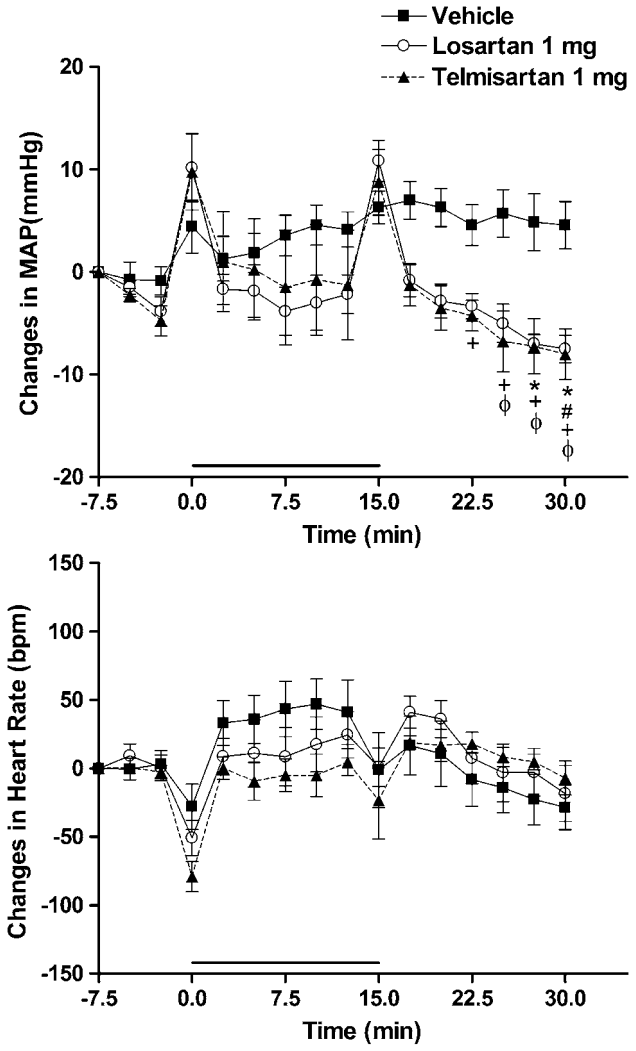

Figure 4 Changes in MAP and HR caused by orthostatic stress (tilt) in conscious rats after oral treatment with losartan (1 mg kg^{-1}, $n=6$), telmisartan (1 mg kg^{-1}, $n=5$) or vehicle (0.9% NaCl, 1 ml kg^{-1}; $n=7$). Values are mean ± s.e.mean. Bar indicates the duration of the maneuvre (0–15 min). *$P<0.05$ losartan vs baseline; $^{\#}P<0.05$ telmisartan vs baseline; $^{+}P<0.01$ losartan vs vehicle; $^{\Phi}P<0.01$ telmisartan vs vehicle. HR, heart rate; MAP, mean arterial pressure.

losartan nor telmisartan significantly altered the HR response to tilting. As observed with the lower dose, a post-tilt hypotension was observed in both oral losartan and telmisartan groups at 10 mg kg^{-1} (mean maximum changes, −13 ± 1 mm Hg losartan vs −9 ± 2 telmisartan; $P<0.01$; Figure 5).

Discussion

In contrast to what was previously reported in anaesthetized rats, the main new finding of this study is that the effect of losartan on the compensatory response of blood pressure during the short-term orthostatic cardiovascular challenge shares a similar profile to that of other highly selective AT_1 antagonist.

The use of antihypertensive drugs might result in orthostatic hypotension, the major cause for orthostatic symptoms (Lamarre-Cliche and Cusson, 2001). Therefore, the development of animal models that might, as closely as possible, reproduce the cardiovascular effects produced by orthostatic challenges would be useful to the investigation of the mechanisms involved. A model for predicting orthostatic hypotension during antihypertensive drug therapy in rats was first reported by Humphrey and McCall (1982) and has been validated by different laboratories (Ohlstein *et al.*, 1992; Hashimoto *et al.*, 1999) including ours (de Moura *et al.*, 2005). However, different anaesthetics might importantly influence the level of peripheral sympathetic outflow or sympathetic reactivity (Weaver and Stein, 1989; Shimokawa *et al.*, 1998), and the sympathetic reactivity is the only mechanism responsible for the maintenance of normal levels

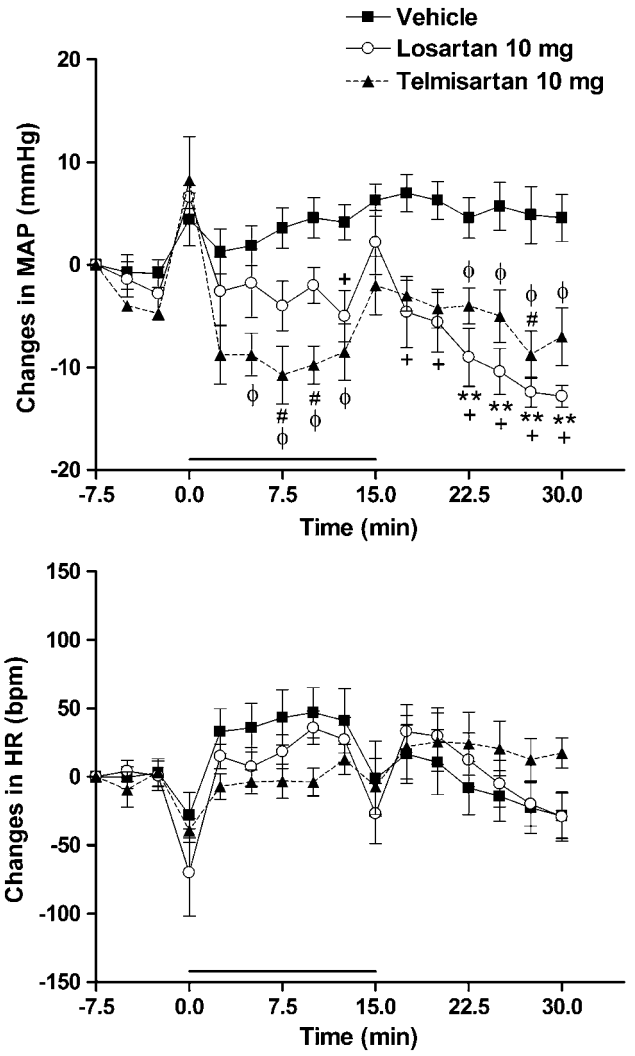

Figure 5 Changes in MAP and HR caused by orthostatic stress (tilt) in conscious rats after oral treatment with losartan (10 mg kg^{-1}, $n=6$), telmisartan (10 mg kg^{-1}, $n=5$) or vehicle (0.9% NaCl, 1 ml kg^{-1}; $n=7$). Values are mean ± s.e.mean. Bar indicates the duration of the maneuvre (0–15 min). $^{**}P<0.01$ losartan vs baseline; $^{\#}P<0.05$ telmisartan vs baseline; $^{+}P<0.01$ losartan vs vehicle; $^{\Phi}P<0.01$ telmisartan vs vehicle. HR, heart rate; MAP, mean arterial pressure.

of blood pressure during short-term orthostatic changes (Goldstein *et al*., 2002; Dampney *et al*., 2003). In conscious dogs, a 40° head-up tilt evokes an immediate increase of 50% in sympathetic activity (Miki *et al*., 1989). In the current study, we used total (α and β) adrenoceptor blockade to validate our method. Prazosin causes orthostatic hypotension by reducing peripheral resistance (Rieckert, 1996) and atenolol reduces the heart output during orthostatic challenges, and both sympathetic-mediated effects are critical to reach normal orthostatic cardiovascular stabilization in the short term (Lamarre-Cliche and Cusson, 2001). Compared with results obtained previously in anaesthetized rats (Humphrey and McCall, 1982; Ohlstein *et al*., 1992), blockade of adrenergic receptors in conscious rats induced a smaller fall in blood pressure during tilt. In the anaesthetized model, only the selective blockade of α_1-adrenergic receptors was sufficient to result in a rapid and extensive fall in blood pressure during tilt, ranging from −20 to −30 mm Hg (Humphrey and McCall, 1982; Ohlstein *et al*., 1992). Curiously, the effect reported with prazosin on blood pressure during tilt in the anaesthetized rat model (Ohlstein *et al*., 1992) is similar in terms of magnitude and time course to the profile of the orthostatic hypotension observed in dysautonomic patients (Lamarre-Cliche and Cusson, 2001). Therefore, the effects of different antihypertensive drugs on the cardiovascular response to orthostatic stress models performed in conscious and anaesthetized preparations might be markedly different.

Previous reports using anaesthetized preparations showed a clear difference between the effects of losartan and other AT_1 antagonists on the cardiovascular response to orthostatic stress, showing a pronounced hypotensive response only in the losartan-treated rats (Ohlstein *et al*., 1992; Hashimoto *et al*., 1999; de Moura *et al*., 2005). In contrast, the present data in conscious rats reveal that the effect produced by losartan on the cardiovascular reactivity to orthostatic stress exhibits a profile similar to that of a highly specific AT_1 antagonist (Maillard *et al*., 2002). Therefore, it is likely that the more pronounced orthostatic hypotensive response described specifically for losartan in the orthostatic stress models reported previously was a consequence of the use of anaesthesia (Ohlstein *et al*., 1992; Hashimoto *et al*., 1999; de Moura *et al*., 2005). Indeed, the current study shows that both antagonists altered the cardiovascular response during tilt when compared with control, but this effect was observed only in the experiments using the highest dose (10 mg kg^{-1}). This finding suggests that the probability of occurrence of orthostatic hypotension after treatment with AT_1 antagonists might depend on the dose of the antagonist.

In this study, we found that both oral losartan and telmisartan groups presented a significant post-tilt hypotension, with both doses used. Based on current data we cannot account for the underlying mechanisms responsible for this effect. Peripheral and central Ang AT_1 receptors are critical to the maintenance of blood pressure levels (Fontes *et al*., 2000; Ferrario, 2006). Therefore, this phenomenon could result from a combination of different mechanisms, for example, diminished peripheral resistance, diminished venous return or altered sympathetic reactivity. The latter hypothesis is particularly interesting since it is known that AT_1 antagonists can easily cross the blood–brain barrier (Li *et al*., 1993; Gohlke *et al*., 2001) and act in brain regions containing sympathetic pre-motor neurons (Fontes *et al*., 1997, 2000; Dampney *et al*., 2002). Therefore, depending on their pharmacological specificity, AT_1 antagonists could differentially interfere, in terms of magnitude, with the reactivity of sympathetic pre-motor neurons, as recently demonstrated in the ventrolateral medulla by Sheriff *et al*. (2006). This central action could result in a different sympathetic reactivity with consequently altered cardiovascular performance to tilting. Certainly, more experiments are necessary to evaluate the involvement of central AT_1 receptors in the cardiovascular response to orthostatic challenge, as well as other mechanisms involved, in conscious rats. Despite that, the post-tilt hypotension observed after losartan and telmisartan in our

experiments might provide an insight into possible clinical relevance. Orthostatic effects and first-dose hypotension are uncommon in losartan recipients, with a reported incidence of $\leqslant$0.5% after 25 or 50-mg dosages (Simpson and McClellan, 2000). However, dizziness is a frequent event reported in both losartan- and telmisartan-treated patients (Goa and Wagstaff, 1996; Simpson and McClellan, 2000; Battershill and Scott, 2006), as it is a common event in patients presenting orthostatic intolerance (Lamarre-Cliche and Cusson, 2001). Therefore, we could speculate that an inability to restore blood pressure promptly to baseline levels after changes in posture might explain the significant incidence of dizziness in patients receiving AT_1 antagonists for hypertension treatment (Goa and Wagstaff, 1996; Simpson and McClellan, 2000; Battershill and Scott, 2006).

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Conflict of interest

The authors state no conflict of interest.

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