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Ventilatory responses to chemoreflex stimulation are not enhanced by angiotensin II in healthy humans

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Abstract

The chemoreflexes exert significant control over respiration and sympathetic outflow. Abnormalities in chemoreflex function may contribute to various disease processes. Based on prior animal studies, we developed the hypothesis that acutely elevating circulating angiotensin II levels into the pathophysiological range increases chemoreflex responsiveness in healthy humans. Eighteen adults were studied before (Pre) and during (Post) low (protocol 1; 2ng/kg/min; n=9) or high (protocol 2; 5 ng/kg/min; n=9) dose angiotensin II infusion (study day 1). Chemoreflex responses were quantified by the pure nitrogen breathing method [slope of the minute ventilation vs. arterial oxygen saturation plot generated during a series (n=10) of 100% inspired nitrogen exposures (1–8 breaths)] and by measuring responses to hypercapnia (7% inspired carbon dioxide). Responses to a non-chemoreflex stimulus were also determined (cold pressor test). Measurements were repeated on a subsequent day (study day 2) before and during infusion of a control vasoconstrictor (phenylephrine) infused at a dose (0.6–1.2 μg/kg/min) sufficient to increase blood pressure to the same degree as that achieved during angiotensin II infusion. We found that despite increasing plasma angiotensin II levels to pathophysiological levels responses to pure nitrogen breathing, hypercapnia, and the cold pressor test were unchanged by low (2 ng/kg/min) and high dose (5 ng/kg/min) angiotensin II infusion (protocols 1 and 2). Similarly, responses measured during phenylephrine infusion (Post) were unchanged (from Pre). These findings indicate that acutely increasing plasma angiotensin II levels to levels observed in disease states, such as human heart failure, does not increase chemoreflex responsiveness in healthy humans.

Introduction

Chemoreflexes exert significant influences over ventilation and sympathetic outflow (Gonzalez et al., 1994; Marshall, 1994; Prabhakar et al., 2004). Chemoreflex function is of

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importance as it may influence the progression of numerous cardiovascular diseases (Prabhakar et al., 2004; Schultz et al., 2007b). Consistent with this suggestion chemoreflex responsiveness is increased in animal models of heart failure (Ding et al., 2009; Ding et al., 2010; Li et al., 2005; Li et al., 2006; Sun et al., 1999a; Sun et al., 1999b) and patients with the disease (Chua et al., 1996a; Chua et al., 1997; Chua et al., 1996b; Di Vanna et al., 2007; Narkiewicz et al., 1999; Ponikowski et al., 2001a; Schultz et al., 2007a). The consequences of enhanced chemoreflex responsiveness in heart failure may include exercise intolerance, as a result of exertional dyspnea (Chua et al., 1996a; Chua et al., 1997; Ciarka et al., 2006) and excess mortality (Giannoni et al., 2009; Ponikowski et al., 2001b). Thus, identification of mechanisms that modulate chemoreflex responsiveness in health, as well as contribute to abnormal function in disease, is of biomedical importance. In this context, angiotensin II (Ang II) may be important.

In *healthy* rabbits acute intravenous infusion of Ang II enhances chemoreflex control over ventilation (Li et al., 2006). This potentiation of chemoreflex responses by Ang II in healthy rabbits occurs rapidly (minutes) after plasma Ang II concentrations are increased to pathophysiological levels (Li et al., 2007; Li et al., 2006). Currently, whether or not Ang II modulates chemoreflex responsiveness in an analogous fashion in humans is unknown. Providing an answer to this basic physiological question would provide important information into the potential role of Ang II as an acute modulator of chemoreflex function in human health and possibly disease. Accordingly, we tested the hypothesis that acutely increasing circulating Ang II levels increases chemoreflex responsiveness in *healthy* humans. To test this hypothesis we employed infusion protocols specifically designed to increase plasma Ang II levels into the pathophysiological range (i.e., to levels observed in human heart failure). Furthermore, to enhance the significance of our findings we employed methods of altering chemoreceptor input that have previously been shown to have prognostic significance in humans (pure nitrogen breathing and hypercapnia) (Giannoni et al., 2009; Ponikowski et al., 2001b).

Materials and Methods

Subjects

A total of 18 male and female volunteers participated in the 2 research protocols associated with this study (Table 1). Inclusion criteria were: 1) age 21–40, 2) healthy (as assessed by history and physical examination), 3) non-smoker, 4) normotensive [blood pressure (BP) at rest <140/90 mmHg], and 5) non-obese (BMI<30 kg/m^2) (Table 1). All volunteers were sedentary to recreationally active. The Institutional Review Board of the Penn State College of Medicine approved the experimental protocols. All experiments were conducted with the understanding and the consent of each subject.

Measurements

BP and Heart Rate—BP was determined non-invasively with a semi-automated device (Dinamap) over the brachial artery and at the fingertip using photoplethysmography (Finometer). Heart rate was determined via ECG.

Respiration and Oxygen Saturation—Minute ventilation, partial end-tidal CO_2, and arterial oxygen saturation (SaO_2) were measured with a respiratory gas monitor (Cardiocap/5 GE Healthcare) via a 2-way non-rebreathing mask and pulse oximeter. The mask covered the subject's nose and mouth and the pulse oximeter was positioned on an earlobe. Both devices were placed on the subject at least 10 minutes before data collection began.

Blood Samples—An intravenous catheter was placed in an antecubital vein of the left arm for administration of study drugs and another intravenous catheter was placed in the right arm for blood sampling. Blood samples were obtained at baseline (>30 minutes after assuming the supine position), 30 minutes after beginning infusions, and after completing the physiological stressors (~90 minutes after beginning the infusions). Plasma Ang II concentrations were quantified via radio-immunoassay (Alpco Diagnostics).

Experimental Design

General experimental protocol—Studies were conducted on supine subjects after a minimum 4-hour fast and 24 hour avoidance of caffeine and alcohol. After instrumenting the subjects, a 30-minute rest period occurred before any measurements were made. Subjects were exposed to 3 physiologic stressors separated by >15 minutes. BP and heart rate were measured in triplicate over the brachial artery before each stressor and throughout each trial on a beat-by-beat basis. Respiratory variables were obtained continuously.

Physiological stressors—Subjects were exposed twice to 3 physiological stressors on 2 separate study days (protocols 1 and 2; see below). The first exposure occurred before (Pre) and the second during (Post) intravenous infusion of Ang II (study day 1) or phenylephrine (study day 2).

Pure Nitrogen Breathing: After a 3-minute baseline, subjects were exposed to a series of 10 periods of 100% nitrogen inhalation (1–8 consecutive breaths). Transitions from room air to nitrogen breathing were accomplished using a 3-way valve positioned at the head of the bed (out of sight of the subject). Consecutive nitrogen exposures were separated by ~2 minutes to allow respiratory parameters to visually return to pre pure nitrogen exposure values. The number of breaths of nitrogen inhalation was varied and randomized to produce a wide range of arterial oxygen desaturations (~75–96%).

Hypercapnia: After a 3-minute baseline, subjects inspired hypercapnic gas (7% carbon dioxide, 30% oxygen, 63% nitrogen) for 3 minutes. Transition to hypercapnic gas was accomplished via a 3-way valve positioned out of the sight of the subject.

Cold Pressor Test: After a 3-minute baseline the subject's right hand was submerged in ice water for 2 minutes. The cold pressor test like the other 2 stressors increases ventilation, but does so via a chemoreflex independent afferent pathway.

Protocol 1: Effect of low dose Ang II on chemoreflex responsiveness—After measuring responses to the physiological stressors (Pre), intravenous infusion of Ang II (2 ng/kg/min; Bachem AG, Switzerland; study day 1) or phenylephrine (0.6–1.2 μg/kg/min;

Baxter Healthcare, Deerfield IL: study day 2) was begun and continued until the end of the study day. The dose of the control vasoconstrictor (phenylephrine) was titrated upward in 0.2 μg/kg/min increments at 3 min intervals until BP approximated levels achieved during the Ang II infusion on study day 1. Thus, infusion occurred in a single blind fashion. Thirty minutes after beginning the infusions the 3 physiological stressors were repeated (Post) in an identical manner to Pre (same order). Infusions were stopped after collection of the last blood sample (~90 min after they started), which occurred after completion of the last physiological stressor. At least 72 hours separated study days 1 and 2. The 2ng/kg/min dose of Ang II was chosen based on pilot studies in which we observed that this dose increases plasma Ang II levels to the lower end of the pathophysiological range in humans.

Protocol 2: Effect of high dose Ang II on chemoreflex responsiveness— Protocol 2 was identical to protocol 1 except the dose of Ang II used for intravenous infusion was 5 ng/kg/min. Measurements made and timing of events were identical between protocols 1 and 2. The 5ng/kg/min dose of Ang II was chosen based on pilot studies in which we observed that this dose increases plasma Ang II levels to the upper end of the pathophysiological range in humans.

Data Collection and Analysis

Physiological data were recorded (MacLab 8e, ADInstruments) at 400 Hz. Responses to hypercapnia and cold pressor test were quantified over 30 second intervals during the stressor. Responses to pure nitrogen breathing were assessed using standard methods as originally described (Edelman et al., 1973). During each pure nitrogen trial (10 trials Pre and 10 trials Post for each subject on each study day), the lowest measured SaO_2 value (nadir) was plotted against the minute ventilation obtained from the 2 largest consecutive breaths after inhalation of 100% nitrogen. These single points (1/trial) were then used to develop a minute ventilation-SaO_2 plot for each subject (Pre and Post) in each protocol (protocol 1 and 2).

Statistical Analysis

The slope of each individual's minute ventilation-SaO_2 plot was determined using linear regression analysis. The slope was used as an index of sensitivity only when the correlation coefficient was found to be significant ($P<0.05$) (Chua et al., 1995; Edelman et al., 1973). Effects of the different interventions were determined using a repeated measure ANOVA. Comparison of subject characteristics between protocols 1 and 2 was determined by unpaired t-tests. Statistical significance was established when $P<0.05$.

Results

Subject Characteristics

Subjects in Protocols 1 and 2 shared similar subject characteristics (Table 1).

Effect of low dose Ang II (Protocol 1)

Low dose Ang II infusion increased ($P<0.05$) plasma Ang II levels ~3-fold (Fig. 1) and increased BP at rest. As expected, increases in BP at rest during phenylephrine infusion

were similar to those during Ang II infusion (Table 2). In contrast, heart rate at rest decreased during phenylephrine infusion, but not Ang II infusion (Table 2). Chemoreflex responsiveness determined during pure nitrogen breathing was unaltered by Ang II and phenylephrine infusion (Fig. 2). The correlation coefficients between minute ventilation and SaO_2 were significant ($P<0.05$) in 8 of 9 subjects. In these 8 subjects the correlation coefficient (r-value) exceeded 0.80 at all conditions/time points (range 0.83–0.86). Similarly, responses to hypercapnia (Table 3 and Fig. 3) and the cold pressor test (Fig. 4) were unaltered by Ang II and phenylephrine infusion. Resting hemodynamic and respiratory parameters (baseline) before each physiological stressor were similar (Table 2).

Effect of high dose Ang II (Protocol 2)

High dose Ang II infusion increased ($P<0.05$) plasma Ang II levels ~5-fold (Fig. 1) and increased BP at rest (Table 2). As expected, increases in plasma Ang II and BP at rest were greater after high versus low dose Ang II infusion (Fig. 1 and Table 2). BP at rest increased similarly during phenylephrine and Ang II infusion. As in protocol 1, heart rate at rest decreased during phenylephrine infusion, but not Ang II infusion (Table 2). Chemoreflex responsiveness determined during pure nitrogen breathing was unaltered by Ang II and phenylephrine infusion (Fig. 2). The correlation coefficients (r-value) between minute ventilation and SaO_2 were significant ($P<0.05$) in 8 of 9 subjects. In these 8 subjects the correlation coefficient exceeded 0.70 at all conditions/time points (range 0.73–0.78). Similarly, responses to hypercapnia (Table 4 and Fig. 3) and the cold pressor test (Fig. 4) were generally unaltered by Ang II or phenylephrine infusion. However, there was a significant drug (Ang II vs. phenylephrine) x condition (Pre vs. Post) interaction observed for absolute heart rate during the hypercapnia and cold pressor test trials (absolute value data not shown), which reflects the downward shift in heart rate observed during phenylephrine infusion that persisted throughout the stressor. As in protocol 1, resting hemodynamic and respiratory parameters before each physiological stressor were similar (Table 2).

Discussion

The present findings indicate that acutely increasing circulating Ang II to levels observed in pathophysiological states, such as human heart failure, does not increase chemoreflex responsiveness to pure nitrogen breathing or hypercapnia in *healthy adults*. These results contrast previous findings in *healthy animals* (rabbits) that demonstrated pronounced rapid increases in chemoreflex responsiveness under very similar experimental conditions (Li et al., 2007; Li et al., 2006). Below we will discuss the significance of the present findings and address discrepancies with prior animal studies.

Previous studies indicate that Ang II increases chemoreflex sensitivity in healthy animals. Specifically, acute intravenous infusion of Ang II in healthy rabbits, at a dose sufficient to produce plasma Ang II levels comparable to those observed in heart failure rabbits, produced rapid and pronounced increases in ventilatory and sympathetic responses to alterations in chemoreflex input (Li et al., 2007; Li et al., 2006). Moreover, in heart failure rabbits, administration of an angiotensin type 1 receptor antagonist decreased chemoreflex responsiveness (Li et al., 2006). The specific mechanism(s) by which Ang II mediates its

effects likely involve signaling processes via the angiotensin type 1 receptor subtype and induction of oxidative stress (Allen, 1998; Li et al., 2007). Collectively, these results strongly suggest that Ang II rapidly and powerfully affect chemoreflex function in both health and disease in animals (i.e., rabbits). However, the present study was unable to translate these prior findings in rabbits to healthy humans despite numerous similarities between the studies, such as duration of Ang II infusion, plasma Ang II levels obtained, and achievement of plasma Ang II levels similar to those observed in heart failure in the respective species.

The stated aim of this study was to determine if acutely increasing plasma Ang II levels to those observed in *human* disease states, such as heart failure, increases measures of chemoreflex responsiveness with prognostic significance in healthy humans. To address this aim we performed 2 separate experimental protocols in which low (protocol 1; 2 ng/kg/min Ang II) and high (protocol 2; 5 ng/kg/min Ang II) doses of Ang II were infused in *healthy adults* to allow examination of responses across the pathophysiological range of Ang II levels (Dzau et al., 1981; MacFadyen et al., 1999; Roig et al., 2000; Swedberg et al., 1990; van de Wal et al., 2006). Effectiveness of our infusion protocols was carefully documented through definitive biochemical measurements of plasma Ang II. Both the present study, and the prior study in healthy rabbits (Li et al., 2006), achieved similar plasma Ang II levels during infusion (~50 pmol/L) that approximate levels observed in disease (i.e., heart failure) in the respective species (Dzau et al., 1981; Li et al., 2006; Liu et al., 2000; MacFadyen et al., 1999; Roig et al., 2000; Swedberg et al., 1990; van de Wal et al., 2006). We acknowledge that the dose of Ang II infused in the present *human* study (2–5 ng/kg/min) was considerably smaller than that in the previous *animal* study (20 ng/kg/min) (Li et al., 2006), but the plasma level of Ang II achieved rather than the dose infused is the critical variable to consider here. However, the fact that a much lower dose of Ang II was able to achieve similar plasma Ang II level in humans versus rabbits does suggest the presence of a species differences in Ang II kinetics.

Developing a better understanding of the mechanisms underlying chemoreflex responsiveness in both health and disease is important. For example, chemoreflex responsiveness is increased in heart failure patients (Chua et al., 1996a; Chua et al., 1997; Chua et al., 1996b; Di Vanna et al., 2007; Narkiewicz et al., 1999; Ponikowski et al., 2001a; Prabhakar et al., 2004; Schultz et al., 2007a; Schultz et al., 2007b) and these increases may contribute to excess morbidity/mortality. A consequence of increased chemoreflex responsiveness in heart failure patients could be greater ventilatory responses to exertion (i.e., exercise) (Chua et al., 1996a; Chua et al., 1997; Ciarka et al., 2006) resulting in excessive exertional dyspnea/exercise intolerance, which are cardinal symptoms in heart failure patients (Chua et al., 1997; Chugh et al., 1996). Additionally, increased chemoreflex responsiveness may contribute to central sleep apnea (Hanly et al., 1993; Wilcox et al., 1993) and arrhythmogenesis (Chua et al., 1997). Collectively, these findings may help explain how increased chemoreflex responsiveness, as measured by a potentiated ventilatory response to chemoreceptor stimulation, helps to identify heart failure patients at elevated risk (Giannoni et al., 2009; Ponikowski et al., 2001b). For these reasons, identification of the mechanism(s) underlying increased ventilatory chemoreflex sensitivity may prove useful in

identifying effective therapies/modalities to reduce significant morbidity/mortality associated with diseases such as heart failure (Roger et al., 2012). Moreover, better understanding of the mechanism underlying normal physiological function in health may allow alterations/abnormalities present in disease (i.e., pathophysiology) to be more readily identified and targeted.

Important interactions between the baroreflexes and chemoreflexes may occur. Studies in humans suggest that when chemoreflex input is altered the baroreflexes reset to operate around a higher level BP, without an apparent effect on their gain (Halliwill et al., 2002; Halliwill et al., 2003; Simmons et al., 2007). Additionally, studies in humans (Somers et al., 1991) suggest that large increases in baroreceptor input impair chemoreflex responsiveness. These findings are consistent with finding in animal studies (Heistad et al., 1975) that indicate that the site of interaction is of central origin (Heistad et al., 1974). These data (Heistad et al., 1975; Heistad et al., 1974; Somers et al., 1991) suggest that our ability to detect increases in chemoreflex responsiveness during Ang II infusion may have been masked by an increase in BP (i.e., baroreceptor activation). However, comparison of responses to Ang II infusion with those obtained on another study day, in which a control vasoconstrictor (phenylephrine) was infused at levels sufficient to approximate the increase in BP observed during Ang II infusion, strongly suggest that a sensitizing effect of Ang II on chemoreflex responsiveness was not masked by a modest increase in BP (i.e., baroreceptor activation). An alternative experimental approach to possibly minimize concerns related to reflex interactions would have been to co-infuse a vasodilator drug with Ang II, in an attempt to avoid changes in baroreceptor input. However, as with all approaches in humans the assumption that changes in baroreceptor input would not occur simply because BP levels were unchanged may not be valid (Taylor et al., 1995).

This study has several limitations. First, chemoreflex control of ventilation and not sympathetic outflow was studied. This was done as the former (Chua et al., 1996a; Li et al., 2005; Ponikowski et al., 2001b), but not the latter (Narkiewicz et al., 1999) is potentiated in human heart failure and carries prognostic significance (Giannoni et al., 2009; Ponikowski et al., 2001b). Second, prior animal data demonstrating potentiation of chemoreflex responses by Ang II were collected under isocapnic hypoxic conditions (Li et al., 2007; Li et al., 2006). The fact that responses to pure nitrogen breathing were not collected under identical conditions may contribute to the divergent findings (Duffin, 2007) in humans and animals. Lastly, the duration of our Ang II infusion was short (~90 min). However, the infusion duration was at least as long as that previously used to demonstrate potentiated chemoreflex responses in healthy rabbits (Li et al., 2006). Future studies could address concerns related to the short exposure to increased circulating levels of Ang II by studying groups that chronically differ in Ang II production and or by measuring responses before and after long-term administration of angiotensin converting enzyme inhibitors or AT1 receptor antagonists.

In conclusion, despite achieving acute increases in plasma Ang II concentrations across the pathophysiological range (~3–5-fold increase from basal levels) we were unable to detect any effect on ventilatory responsiveness to several distinct chemoreflex stimuli with prognostic significance (Giannoni et al., 2009; Ponikowski et al., 2001b) in healthy humans.

These data contrast prior animal studies, which demonstrate rapid and pronounced increases in chemoreflex sensitivity when plasma Ang II levels are raised to nearly identical levels.

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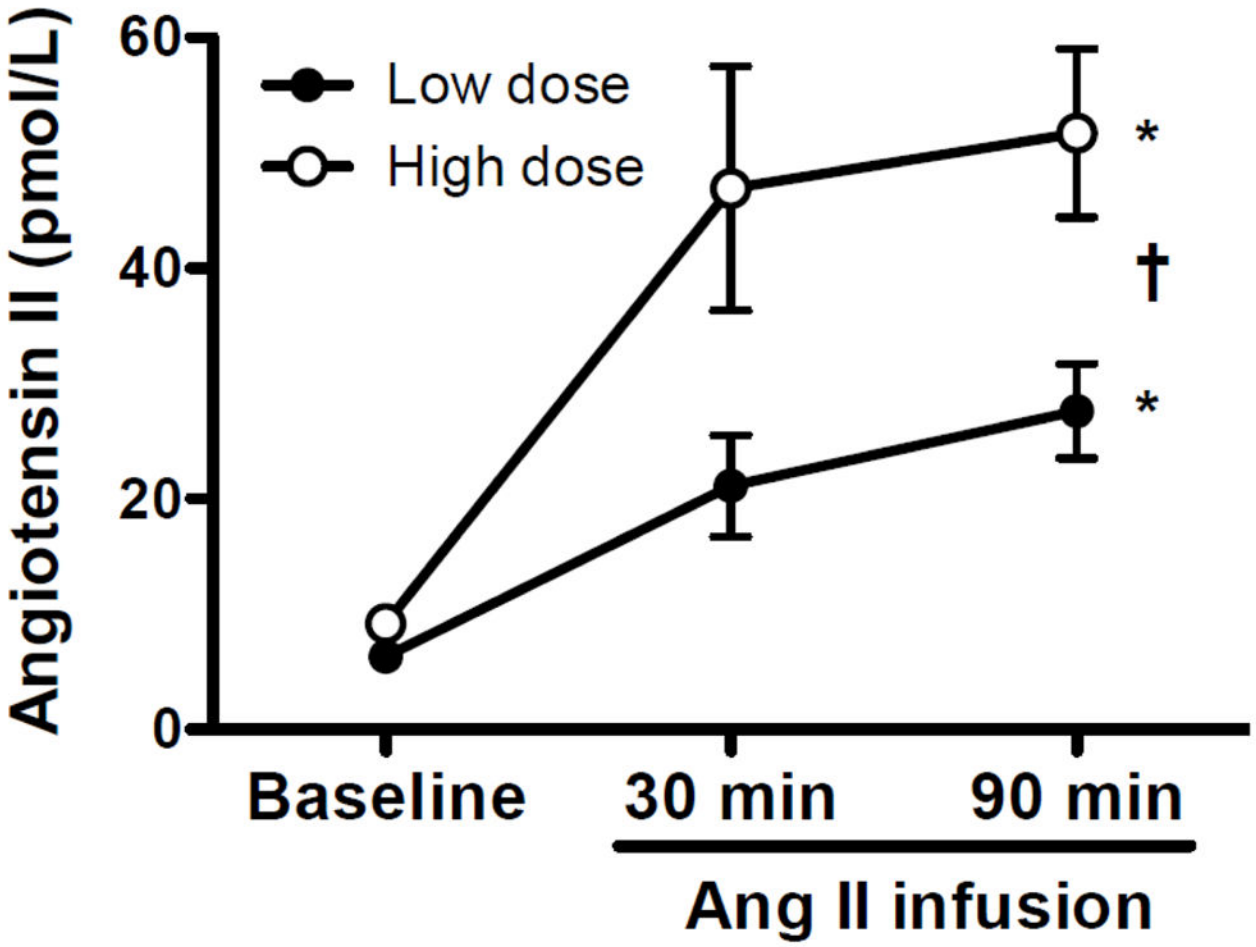

Figure 1.

Plasma angiotensin II (Ang II) levels measured before (Baseline) and 30 and ~90 min after beginning intravenous infusion of low (2 ng/kg/min Ang II; closed symbols; protocol 1) or high dose Ang II (5 ng/kg/min; open symbol; protocol 2). Plasma Ang II levels were similar at baseline (pre-infusion) in both groups and increased in a time- (* $P<0.05$ time main effect) and dose-dependent fashion († $P<0.05$ dose x time interaction) with infusion. Values are mean±SE.

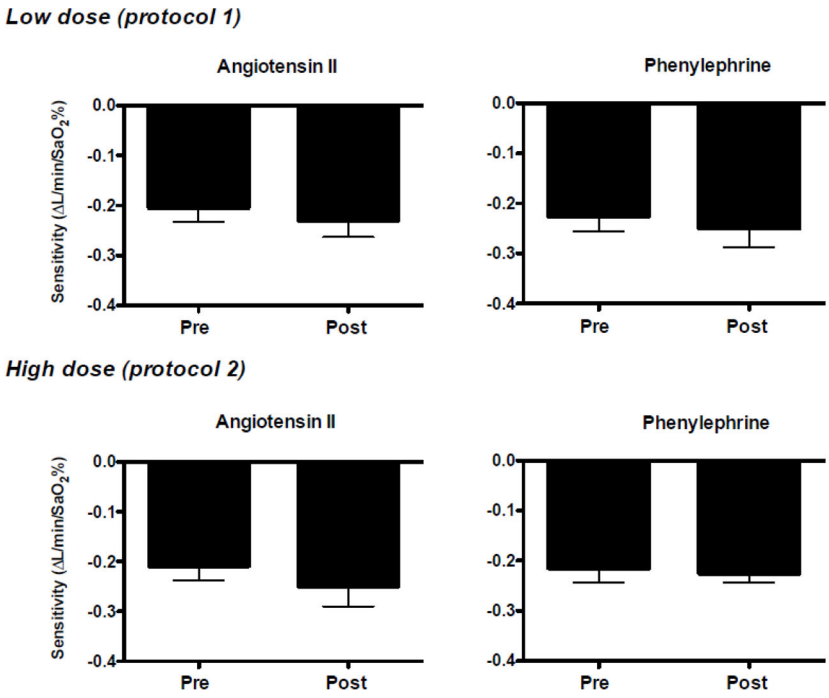

Figure 2.

Responses to pure nitrogen breathing measured before (Pre) and during (Post) low (upper panels) or high dose (lower panels) angiotensin II (left panels) and phenylephrine infusion (right panels). Subjects were exposed to a series of 10 trials of 100% inspired nitrogen (1–8 breaths). Consecutive pure nitrogen trials were separated by ~ 2 min. For analysis, after each nitrogen exposure the nadir in arterial oxygen saturation (SaO_2) was obtained and plotted as a function of the minute ventilation of the 2 largest consecutive breaths to develop a minute ventilation-SaO_2 plot. The slope of this plot was used as an index of sensitivity. Neither low nor high dose angiotensin II or phenylephrine infusion had an effect on sensitivity. Values are mean±SE.

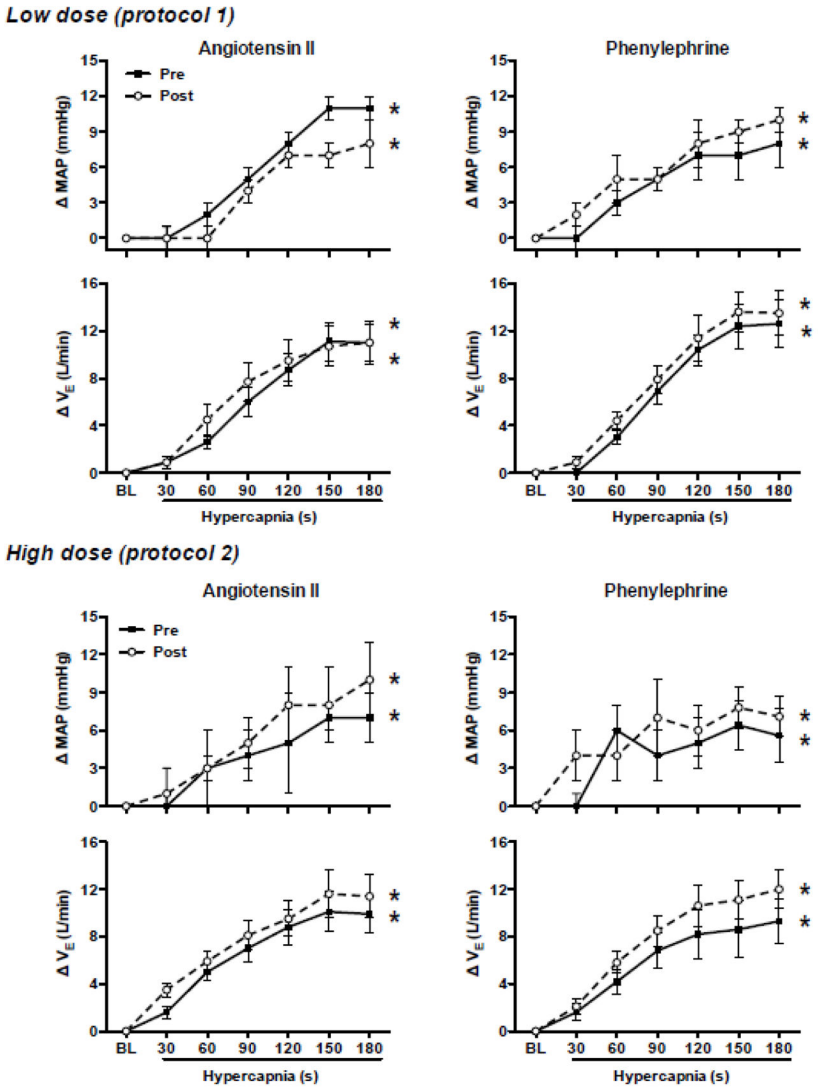

Figure 3.

Mean arterial blood pressure (MAP) and minute ventilation (V_E) responses to hypercapnia (7% inspired carbon dioxide). Responses are presented as change from baseline levels (room air breathing) in protocol 1 (upper panels) and 2 (lower panels) before (Pre; closed symbols) and during (Post; open symbols) angiotensin II (left panels) or phenylephrine (right panels) infusion. Data are presented as 30 s mean values in response to the 3 min exposure. Both MAP and V_E increased during hypercapnia. However, responses were not altered by infusion of low or high dose angiotensin II or phenylephrine. Values are mean±SE.

* $P<0.05$ time main effect

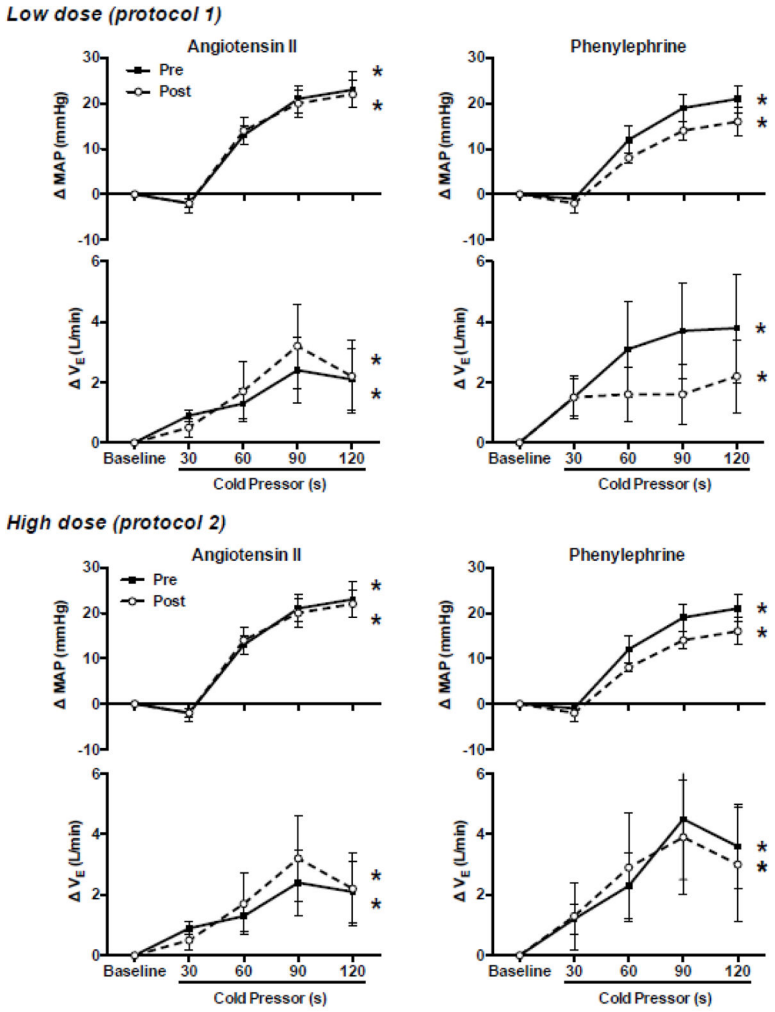

Figure 4.

Mean arterial blood pressure (MAP) and minute ventilation (V_E) responses to the cold pressor test (placing hand in ice water). Responses are presented as change from baseline levels in protocol 1 (upper panels) and 2 (lower panels) before (Pre; closed symbols) and during (Post; open symbols) angiotensin II (left panels) or phenylephrine (right panels) infusion. Data are presented as 30 s mean values in response to the 2 min exposure. Both MAP and V_E increased during the cold pressor test. However, responses were not altered by infusion of low or high dose angiotensin II or phenylephrine. Values are mean±SE.

* $P<0.05$ time main effect

Table 1

Subject characteristics

	Protocol 1 (n=9)	Protocol 2 (n=9)
Sex	5 male/4 female	5 male/4 female
Age, years	27±1	26±1
Height, cm	181.8±3.2	174.3±3.2
Body mass, kg	78.4±4.7	76.4±4.0
BMI, kg/m^2	23.6±0.9	25.0±0.8
Systolic BP, mmHg	117±3	118±2
Diastolic BP, mmHg	65±2	64±2
Mean BP, mmHg	86±1	86±1
Heart rate, bt/min	57±3	56±3

Values are mean±SE

BMI = body mass index; BP = blood pressure

Table 2

Hemodynamic and respiratory parameters at rest before each physiological stressor

	Protocol 1 (n=9)				Protocol 2 (n=9)			
	Angiotensin II		Phenylephrine		Angiotensin II		Phenylephrine	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Pure nitrogen breathing								
Mean BP, mmHg	87±1	92±1*	86±1	91±1*	86±1	98±2*	86±1	95±2*
Heart rate, bt/min	55±3	56±3	57±4	50±3*†	56±3	57±4	55±3	47±3*†
V_E, L/min	5.8±0.5	6.2±0.5	6.3±0.5	6.3±0.5	5.5±0.4	5.9±0.4	5.9±0.5	6.4±0.4*
SaO_2, %	98±1	98±1	99±1	98±1	98±1	98±1	99±1	99±1
Hypercapnia								
Mean BP, mmHg	85±2	91±1*	85±1	91±1*	87±1	95±2*	86±1	94±2*
Heart rate, bt/min	61±3	60±3	66±4	53±3*†	58±4	59±4	59±2	49±3*†
V_E, L/min	6.1±0.3	5.9±0.4	6.7±0.6	6.6±0.5	5.3±0.5	6.0±0.3	6.1±0.5	6.4±0.6
SaO_2, %	99±1	99±1	98±1	98±1	98±1	98±1	99±1	98±1
Cold pressor rest								
Mean BP, mmHg	86±2	91±1*	86±1	91±1*	86±1	97±2*	86±1	94±2*
Heart rate, bt/min	62±3	60±3	62±4	52±3*†	61±4	63±5	59±3	51±3*†
V_E, L/min	6.7±0.6	6.7±0.4	6.7±0.5	6.8±0.6	6.1±0.4	6.5±0.4	6.3±0.5	6.9±0.6*
SaO_2, %	99±1	98±1	98±1	98±1	99±1	98±1	99±1	99±1

Pre measures were made each study day *before* angiotensin II (2 or 5 ng/kg/min for protocols 1 and 2, respectively) or phenylephrine infusion (0.6–1.2 μg/kg/min). Post measurements were made at least 30 minutes after starting angiotensin II or phenylephrine infusion.

Values are mean±SE.

BP = blood pressure; V_E = minute ventilation; SaO_2 = arterial oxygen saturation

*P<0.05 vs. Pre;

†P<0.05 drug (angiotensin II vs. phenylephrine) x condition (pre vs. post) interaction

Table 3

Hemodynamic and respiratory responses to hypercapnia (protocol 1; n=9)

		Hypercapnia					
	Baseline	**30**	**60**	**90**	**120**	**150**	**180**
Angiotensin II							
Pre							
Heart rate, bt/min	61±3	63±3	63±3	66±3	68±4	67±4	68±4†
Mean BP, mmHg	85±2	86±2	87±2	90±2	94±2	96±2	96±2†
V_E, L/min	6.1±0.3	6.7±0.7	8.7±0.6	12.1±1.2	14.8±1.5	17.2±1.8	17.1±1.8†
$PetCO_2$, mmHg	45±1	54±1	56±1	57±1	58±1	59±1	59±1†
Post							
Heart rate, bt/min	60±3	64±3	64±3	65±3	61±3	62±3	62±3
Mean BP, mmHg	91±1*	91±1	91±1	95±1	98±2	98±2	99±2†
V_E, L/min	5.9±0.4	6.8±0.5	10.5±1.4	13.6±1.7	15.4±1.8	16.6±1.9	16.9±2.1†
$PetCO_2$, mmHg	44±1	54±1	59±2	59±2	59±2	58±1	59±1†
Phenylephrine							
Pre							
Heart rate, bt/min	65±4	67±4	67±4	70±4	71±3	71±3	71±4†
Mean BP, mmHg	85±1	85±2	88±1	91±2	92±2	92±2	93±2†
V_E, L/min	6.7±0.6	6.5±0.5	9.7±0.6	13.6±1.0	17.0±1.5	19.0±2.0	19.3±2.1†
$PetCO_2$, mmHg	44±1	53±1	56±2	57±1	57±1	58±1	58±1†
Post							
Heart rate, bt/min	53±3*‡	53±3	53±4	56±4	57±3	59±3	60±3†
Mean BP, mmHg	91±1*	93±1	96±2	87±2	98±2	100±2	100±2†
V_E, L/min	6.6±0.5	7.5±0.5	11.0±0.9	14.5±1.3	17.9±2.1	20.2±1.9	20.0±22†
$PetCO_2$, mmHg	45±1	55±1	57±2	58±1	58±1	59±1	59±1†

Responses to hypercapnia (7% inspired carbon dioxide) measured before (Pre) and during (Post) low dose angiotensin II (2 ng/kg/min) or phenylephrine infusion (0.6–1.2 μg/kg/min). Post measurements were made at least 30 minutes after starting angiotensin II or phenylephrine infusion. Values were determined in the 3 min period before hypercapnia (Baseline) as well as at 30 s intervals during hypercapnia. Subjects were exposed to both infusions on separate study days. Responses measured Pre and Post were similar on both study days.

Values are mean±SE.

BP = blood pressure; V_E = minute ventilation; $PetCO_2$ = end tidal carbon dioxide

* P<0.05 vs. Baseline (Pre);

† P<0.05 time main effect;

‡ P<0.05 drug (angiotensin II vs. phenylephrine) x condition (pre vs. post) interaction (Baseline)

Table 4

Hemodynamic and respiratory responses to hypercapnia (protocol 2; n=9)

		Hypercapnia					
	Baseline	**30**	**60**	**90**	**120**	**150**	**180**
Angiotensin II							
Pre							
Heart rate, bt/min	58±4	63±5	63±5	63±4	64±4	63±4	63±4†
Mean BP, mmHg	87±1	87±2	90±2	91±2	92±2	94±2	94±2†
V_E, L/min	5.3±0.5	6.9±0.7	10.3±1.1	12.3±1.5	14.2±2.0	15.4±2.1	15.2±2.1†
$PetCO_2$, mmHg	46±2	56±1	58±1	58±1	62±2	60±1	60±2†
Post							
Heart rate, bt/min	59±4	63±4	63±4	64±4	64±4	63±4	63±3†§
Mean BP, mmHg	95±2*	95±2	98±2	100±2	103±2	102±2	104±2†
V_E, L/min	6.0±0.3	6.8±0.5	10.5±1.4	13.6±1.7	15.4±1.8	16.6±1.9	16.9±2.1†
$PetCO_2$, mmHg	46±3	55±1	59±3	57±1	58±2	58±1	58±1†
Phenylephrine							
Pre							
Heart rate, bt/min	59±2	62±3	62±3	62±3	63±4	64±3	65±3†
Mean BP, mmHg	86±1	86±1	89±2	90±2	91±2	92±2	92±2†
V_E, L/min	6.1±0.5	7.6±0.8	10.3±1.1	12.9±1.6	14.2±2.1	14.7±2.5	15.3±1.9†
$PetCO_2$, mmHg	45±1	55±2	56±2	57±2	58±1	59±1	59±1†
Post							
Heart rate, bt/min	49±3*‡	52±3	52±3	53±3	56±3	57±4	58±4†§
Mean BP, mmHg	94±2*	99±3	99±3	101±4	100±3	102±2	102±2†
V_E, L/min	6.4±0.6	8.4±1.0	12.1±1.1	14.8±1.5	17.0±2.0	17.5±1.8	18.3±1.9†
$PetCO_2$, mmHg	44±1	56±1	57±1	58±1	58±1	59±1	61±2†

Responses to hypercapnia (7% inspired carbon dioxide) measured before (Pre) and during (Post) high dose angiotensin II (5 ng/kg/min) or phenylephrine infusion (0.6–1.2 μg/kg/min). Post measurements were made at least 30 minutes after starting angiotensin II or phenylephrine infusion. Values were determined in the 3 min period before hypercapnia (Baseline) as well as at 30 s intervals during hypercapnia. Subjects were exposed to both infusions on separate study days. Responses measured Pre and Post were similar on both study days.

Values are mean±SE.

BP = blood pressure; V_E = minute ventilation; $PetCO_2$ = end tidal carbon dioxide

* P<0.05 vs. Baseline (Pre);

† P<0.05 time main effect;

‡ P<0.05 drug (angiotensin II vs. phenylephrine) x condition (pre vs. post) interaction (Baseline);

§ P<0.05 drug (angiotensin II vs. phenylephrine) x condition (pre vs. post) interaction