Effects of angiotensin II on autonomic components of nasopharyngeal stimulation in male conscious rabbits

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Submitted 24 November 2004; accepted in final form 7 January 2005

Mousa, Tarek M., Lie Gao, Kurtis G. Cornish, and Irving H. Zucker. Effects of angiotensin II on autonomic components of nasopharyngeal stimulation in male conscious rabbits. J Appl Physiol 98: 1607–1611, 2005. First published January 13, 2005; doi:10.1152/japplphysiol.01322.2004.—Angiotensin II (ANG II) is known to activate central sympathetic neurons. In this study we determined the effects of ANG II on the autonomic components of the cardiovascular responses to stimulation of nasopharyngeal receptors with cigarette smoke. Experiments were carried out in conscious New Zealand White rabbits instrumented to record arterial pressure and heart rate. Rabbits were exposed to 50 ml of cigarette smoke before and after subcutaneous osmotic minipump delivery of ANG II at a dose of 50 ng·kg⁻¹·min⁻¹ for 1 wk in one group and intracerebroventricular (icv) infusion at a dose of 100 pmol/min for 1 h in a second group. The responses were compared before and after heart rate was controlled by pacing. Autonomic components were evaluated by intravenous administration of atropine methyl bromide (0.2 mg/kg) and prazosin (0.5 mg/kg). ANG II given either systemically or icv significantly blunted the pressor response to smoke (P < 0.05) when the bradycardic response was prevented. This blunted response was not due to an absolute increase in baseline blood pressure after ANG II infusion (71.64 ± 11.6 vs. 92.1 ± 19.8 mmHg; P < 0.05) because normalization of blood pressure with sodium nitroprusside to pre-ANG II levels also resulted in a significantly blunted pressor response to smoke. The effect of smoke was α-adrenergic receptor-mediated because it was essentially abolished by prazosin in both the pre- and the post-ANG II states (P < 0.05). These results suggest that elevations in central ANG II reduce the sympathetic response to smoke in conscious rabbits. This effect may be due to an augmentation of baseline sympathetic outflow and a reduction in reflex sensitivity similar to the effect of ANG II on baroreflex function.

Nasopharyngeal stimulation with cigarette smoke is reported to produce dramatic cardiovascular and respiratory responses in rabbits (31, 33). These include a rise in blood pressure, profound bradycardia, and apnea. The cardiovascular responses are initiated mainly in trigeminal nerve endings and are mediated by reflex trigeminal excitation of both sympathetic and cardiac vagal efferents, accounting for the increase in blood pressure and for the bradycardia seen in response to smoke, respectively (31, 33).

This cardiovascular reflex, which is caused by sympathetic vasoconstriction and cardiovagal excitation, is thought to act to maintain and utilize limited oxygen stores via the preferential redistribution of blood flow to the brain and heart during the reflex apnea (7, 32). Similar responses are seen during immersion of the face in water, the so-called diving or bathytropic reflex, to reduce total oxygen consumption during the period of hypoxia. Additionally, neural inputs from the nasopharynx can augment baroreflex function in the central nervous system (3, 4, 24). Although ANG II is known to inhibit arterial baroreflex function, there is evidence of other reflex alterations, such as the cardiopulmonary reflex (30) by ANG II; however, the effect on the nasopharyngeal reflex has not been previously tested. In this study, we hypothesized that ANG II would inhibit the reflex effects of nasopharyngeal stimulation with cigarette smoke.

Therefore, the objective of the current work was to study the effect of ANG II on the autonomic components (sympathetic and parasympathetic) of the cardiovascular responses to stimulation of nasopharyngeal receptors with cigarette smoke in conscious rabbits. As a comparison, the role of ANG II in affecting arterial baroreflex function was also investigated.

METHODS

Animals. Experiments were carried out on male New Zealand White rabbits (Harlan, Indianapolis, IN) ranging in weight between 3 and 4 kg. All experiments were approved by the University of Nebraska Medical Center Animal Care and Use Committee and conformed to the Guidelines for Care and Use of Laboratory Animals of the American Physiological Society and the National Institutes of Health.

Chronic instrumentation. All rabbits underwent sterile thoracic instrumentation as described previously (19). With the animals under general anesthesia, a left thoracotomy was performed in the third intercostal space. After the pericardium was opened, a platinum-wire pacing electrode was sutured to the epicardium of the left ventricle. A reference electrode was secured to the left atrium. All wires were tunneled beneath the skin and exited in the midscapular area. The chest was closed and evacuated. At the same surgery, a radiotelemetry transducer and catheter (ADInstruments) were implanted into the right femoral artery. The tip of the catheter was positioned in the abdominal aorta to monitor the pulsatile and mean arterial blood pressure (MAP) and heart rate (HR) by using Data Sciences and ADInstruments Powerlab data acquisition systems.

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group, we studied the effect of central ANG II on the autonomic components of the response to the same smoke stimulus.

Muscarinic receptor blockade was accomplished by giving atropine methyl bromide (0.2 mg/kg) intravenously through a lateral ear vein. On a separate day, the effect of α₁-adrenergic receptor blockade was examined by using intravenous prazosin (0.5 mg/kg). Because a profound bradycardia was seen in response to smoke HR was controlled using an external pacemaker adjusted to a rate just above the resting HR of the rabbit.

Responses to smoke were repeated two to three times separated by at least 5 min. The response of a given rabbit was the average of these responses. Baseline values and peak responses to nasopharyngeal smoke stimulation were compared (change in MAP) after the various interventions.

Arterial baroreflex control of HR was also determined in response to intravenous injections of 100 μg·kg⁻¹·min⁻¹ sodium nitroprusside (SNP) and to 80 μg·kg⁻¹·min⁻¹ of phenylephrine (PE) 10 min after smoke response assessment. Arterial baroreflex curves were constructed as previously described by our laboratory (17, 18).

In brief, several points for HR were taken during the fall or rise in arterial pressure after the administration of SNP and PE. Care was taken to ensure that the blood pressure change was ~1–2 mmHg/s. Data points were obtained at 2-s intervals. The slope of the linear regression between MAP and HR was taken as the baroreflex sensitivity.

**Statistical analysis.** Data are expressed as means ± SE. A two tailed paired t-test for responses within groups was used. Differences between groups were determined by a one-way ANOVA for repeated measures. Post hoc analysis consisted of the Bonferroni test. A probability value of *P* < 0.05 was considered statistically significant.

RESULTS

Original recordings of blood pressure and HR in a conscious rabbit and its responses to nasopharyngeal stimulation with cigarette smoke are shown in Fig. 1. An abrupt fall in HR in response to smoke with little or no increase in blood pressure was seen in rabbits where HR was not controlled (Fig. 1A). On the other hand, when HR was controlled and the bradycardia was prevented smoke elicited a profound increase in arterial pressure (Fig. 1B). Muscarinic receptor blockade (0.2 mg/kg
Table 1. Baseline mean arterial blood pressure and heart rate before smoke stimulation in conscious rabbits

<table>
<thead>
<tr>
<th></th>
<th>Before iv ANG II (n = 6)</th>
<th>After iv ANG II (n = 6)</th>
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<tbody>
<tr>
<td>Map, mmHg</td>
<td>HR, beats/min</td>
<td></td>
</tr>
<tr>
<td>HR uncontrolled</td>
<td>73.1 ± 4.7</td>
<td>93.2 ± 7.3*</td>
</tr>
<tr>
<td>HR controlled</td>
<td>72.9 ± 4.9</td>
<td>84.3 ± 5.1*</td>
</tr>
<tr>
<td>Atropine</td>
<td>77.2 ± 3.2</td>
<td>86.5 ± 6.4*</td>
</tr>
<tr>
<td>Prazosin with HR controlled</td>
<td>60.0 ± 4.3</td>
<td>51.8 ± 7.8</td>
</tr>
</tbody>
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Values are means ± SE; n, no. of animals. ANG II, angiotensin II; MAP, mean arterial blood pressure; HR, heart rate; iv, intravenous. *P < 0.05 compared with pre ANG II.

Table 2. Baseline mean arterial blood pressure and heart rate before and after icv infusion of ANG II in conscious rabbits

<table>
<thead>
<tr>
<th></th>
<th>MAP, mmHg</th>
<th>HR, beats/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before icv ANG II</td>
<td>60.9 ± 3.8</td>
<td>240.0 ± 0</td>
</tr>
<tr>
<td>After icv ANG II</td>
<td>78.4 ± 5.9*</td>
<td>240.0 ± 0</td>
</tr>
<tr>
<td>After icv ANG II + SNP</td>
<td>61.5 ± 4.9</td>
<td>257.3 ± 6.8</td>
</tr>
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</table>

Values are means ± SE. icv, Intracerebroventricular; SNP, sodium nitroprusside. *P < 0.01 compared with pre ANG II.

Atropine methyl bromide (atropine methyl bromide) resulted in abolishment of the bradycardic response to smoke and a resultant significant increase (P < 0.001; Fig. 2) in blood pressure in response to smoke. The pressor response seen either after atropine or during cardiac pacing represents the sympathetic component of the reflex because it was abolished by \( \alpha_1 \)-adrenergic blockade (P < 0.001 comparing before vs. after blockade) by an intravenous dose of 0.5 mg/kg prazosin (Fig. 2).

Role of angiotensin. The role of chronic ANG II infusion on the components of the smoke reflex was investigated in the same rabbits used to determine the autonomic components before ANG II. Systemic ANG II infusion for 1 wk (50 ng·kg\(^{-1}\)·min\(^{-1}\)) resulted in a significant increase in baseline blood pressure which was not affected by keeping HR constant or by preventing the bradycardia using atropine (Table 1). The response to smoke while controlling HR was significantly blunted (P < 0.05) after the 1 wk of systemic ANG II infusion (Fig. 2).

At the same time, depressed baroreflex control of HR after systemic ANG II infusion was observed (P < 0.05) for both the SNP and the PE responses as shown in Fig. 3.

To determine whether the inhibition of the nasopharyngeal reflex by systemic ANG II could be elicited by central administration of ANG II, we administered an icv infusion of ANG II and examined the response to smoke. Short-term experiments were carried out to study the effect of icv ANG II for 1 h (100 pmol/h) on the sympathetic component of the nasopharyngeal reflex while keeping the HR controlled by means of an external pacemaker. As shown in Table 2, icv ANG II significantly elevated baseline blood pressure (P < 0.01) This

infusion blunted the sympathetic response to smoke (P < 0.001), as shown in Fig. 4.

There is a possibility that this blunted response to smoke by ANG II might be because the baseline blood pressure was higher following ANG II. Therefore, we studied the effect of central icv ANG II on the sympathetic component of the nasopharyngeal reflex after normalizing arterial pressure to the levels seen before icv ANG II by concurrent intravenous infusion of SNP (see Table 2). Again, the response was significantly blunted (P < 0.001; Fig. 4).

DISCUSSION

The purpose of this study was to determine the effect of ANG II on the autonomic components of the cardiovascular responses to stimulation of oropharyngeal receptors with cigarette smoke in conscious rabbits. This reflex is similar to the breath-holding diving reflex, which is thought to play a defensive role in protecting against hypoxia by conserving oxygen (1). This is the first study to document the cardiovascular autonomic components to nasopharyngeal stimulation with cigarette smoke in the face of ANG II infusion in conscious rabbits.

We do not believe that the effect of smoke is due to the effect of the nicotine content of the cigarette smoke, because in a study by Kobayashi et al. (14), similar responses (profound bradycardia) were observed by exposing chloralose- and urethane-anesthetized rats to noncigarette smoke using instead "burnt tissue paper" as their nasopharyngeal stimulant. Moreover, in another study by Bogdanowicz et al. (2), it was shown that even stimulation of the upper respiratory tract with "water" evoked marked bradycardia in anesthetized rats. However, nicotine might indeed be playing a role in evoking this reflex as has been proposed by Hartiala et al. (12) in anesthetized dogs. In the latter study, however, the investigators examined
smoke-induced bronchoconstriction rather than a cardiovascular response.

Muscarinic receptor blockade prevented the profound bradycardia in response to smoke stimulation. This observation indicates that the bradycardic component of this reflex is mediated through a parasympathetic, cardiac vagal effect. This result is consistent with similar results observed earlier by White et al. (32) and shown to exist for the diving reflex (1, 16). The fact that intravenous prazosin abolished the pressor response seen when HR was controlled indicates that this pressor response is mediated by a sympathetic α-adrenergic mechanism. These data are consistent with the findings of Nakamura and Hayashida (20) in which they observed a 4-fold increase in RSNA and a 4.4-fold increase in plasma norepinephrine 1 min after the peak response to smoke exposure. In an interesting study by Peterson et al. (22), a 62% decrease in cardiac sympathetic nerve activity was observed, whereas a 248% increase in RSNA was seen during simultaneous recordings in anesthetized rabbits. Heterogeneity in sympathetic outflow cannot be determined in the present study.

Our findings are similar those of Fletcher (9). In his study, there was no significant difference in the bradycardic response to nasopharyngeal smoke stimulation between normotensive and renal hypertensive female rabbits induced by cellophane wrapping of left kidney followed by right nephrectomy. In our study, chronic systemic ANG II infusion had no significant effect on the bradycardic response to nasopharyngeal smoke stimulation (Fig. 2). Fletcher also observed an associated blunted baroreflex sensitivity to methoxamine and nitroglycerine. Nevertheless, the MAP increase in his model was much higher compared with what we observed after systemic ANG II infusion. In Fletcher’s study, MAP reached 137 ± 2 and 147 ± 5 mmHg at 6 and 12 wk, respectively, whereas we observed a modest, yet significant, increase of MAP (93.2 ± 7.3 mmHg). Moreover, we believe that if Fletcher had examined the same response while controlling HR, he would have elicited a similar blunted response as in our study.

Chronic systemic ANG II infusion blunted the pressor response to smoke when the HR was controlled, preventing the bradycardic response from ensuing. This effect was thought to be due to a central action of ANG II at specific foci in the brain, although these loci have not been defined in this study. To show that central ANG II is capable of inhibiting the response to nasopharyngeal stimulation, we conducted experiments in which icv ANG II was administered.

The increased baseline blood pressure seen in response to icv ANG II infusion was not the cause of the blunted pressor response because “normalizing” the blood pressure to the pre-ANG II levels by concurrent infusion of SNP also resulted in a blunted pressor response to smoke that was, in fact, even more pronounced than before SNP. This might be attributed to the possibility that the baseline sympathetic outflow was increased in the face of the SNP infusion, although this is speculative.

The implication of these data to disease states relates to the association between a blunted baroreflex function and an overexpression of ANG II type 1 receptor protein and message in specific areas of the medulla and hypothalamus in the heart failure state in which there is increased circulating levels of ANG II (35, 36). Central ANG II infusion for 1 h resulted in a blunted sympathetic response to nasopharyngeal stimulation similar to that seen with systemic ANG II infusion for 1 wk. Arterial baroreflex control of HR was depressed at the same time as the sympathetic response to nasopharyngeal stimulation. This aroused the assumption of a common central mechanism of inhibition by ANG II to both reflexes or of an effect on a common central sympathetic regulatory outflow site, the rostral portion of the ventrolateral medulla. In addition it is possible that the paraventricular nucleus (PVN) is involved in this response. The PVN is located in the hypothalamus and it is a central site, which is known to be involved in autonomic and neuroendocrine regulation of central sympathetic outflow (28, 29). Neurons from the PVN project to several regions in the central nervous system, including the nucleus of tractus solitarius (26, 27), the rostral ventrolateral medulla (6, 23, 34), and the intermediolateral cell column of the spinal cord (5, 13). These structures are all known to be important in the regulation of sympathetic outflow. The PVN is also richly endowed with ANG II type 1 receptors (36).

In summary, these results suggest that elevations in central ANG II reduce the sympathetic response to smoke inhalation in conscious rabbits. This effect may be due to an augmentation of baseline sympathetic outflow and a reduction in reflex sensitivity similar to the effect of ANG II on baroreflex function. The precise location of the action of ANG II is not known. These data have relevance to the role of ANG II in alterations of cardiovascular reflex activity and sympathetic tone in disease states such as heart failure, hypertension, and diabetes.

GRANTS

This study was supported, in part, by National Heart, Lung, and Blood Institute Grant PO-1 HL-62222.

REFERENCES


