Separation of peripheral and central cardiovascular actions of angiotensin II

Christopher J. Mathias.

Separation of peripheral and central cardiovascular actions of angiotensin II. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H2620–H2626, 1997.—The pressor and vasoconstrictor action of angiotensin II (ANG II) is considered to be caused by a combination of its direct and indirect vascular effects, the latter mediated by the sympathetic nervous system. The purpose of this study was to determine the extent to which the direct and indirect actions of ANG II contribute to its pressor and vascular effects. Blood pressure, cutaneous vascular, and plasma norepinephrine responses to intravenous ANG II were measured in conscious rabbits before and after inhibition of central sympathetic outflow with intravenous and intracisternal clonidine and after ganglionic blockade with intravenous pentolinium. Intravenous ANG II caused a similar dose-related rise in blood pressure before and after sympathetic blockade with intravenous clonidine, intracisternal clonidine, and intravenous pentolinium. In contrast, the dose-related fall in cutaneous ear blood flow and cutaneous ear temperature and rise in cutaneous ear vascular resistance induced by intravenous ANG II were abolished after intravenous clonidine, intracisternal clonidine, and intravenous pentolinium. Heart rate was unchanged after ANG II. There were no changes in body weight or temperature. There was a nonsignificant fall in plasma norepinephrine and no change in epinephrine after ANG II. These results demonstrate that the acute pressor response to intravenous ANG II is mediated by its direct vascular effects and is not dependent on central or ganglionic stimulation of the sympathetic nervous system, in contrast to the pressor effects of ANG II on cutaneous ear vasconstriction, which is predominantly caused by a centrally mediated increase in sympathetic nervous activity. Our results separate, in conscious rabbits, the direct vascular effects of ANG II from its indirect vascular actions, which are mediated by central sympathetic stimulation in the brain.

Methods

Experiments were performed on seven male Sandy Half-Lop rabbits (National Institute for Medical Research, London, UK) with free access to food (RHM-R14, Labure Animal Diets) and water. Animals weighed between 2.8 and 3.4 kg.

Surgical procedure. A cisternal catheter was inserted into the cisterna magna under Saffan general anesthesia as described previously (22). Injections were given in 25 µl of sterile, nonpyrogenic, isotonic saline using a sterilized Hamilton syringe with a 30-gauge needle. This was flushed with 20 µl of saline; the dead volume of the catheter was 10 µl. The injection site was confirmed at the end of a series of experiments by injection of 25 µl of bromophenol blue (1%) followed by 20 µl of saline. The animal was killed with an overdose of pentobarbital sodium, and the distribution of dye was examined.

Experimental protocol. Animals were not used for at least 7 days postsurgery. By this time, parameters of heart rate, blood pressure, respiration rate, and plasma norepinephrine and epinephrine have returned to normal levels. Before each experiment cannulas were inserted into the central artery and marginal vein of the ear under 1% lidocaine (International Medical Systems) local anesthesia. The arterial cannula was connected to a pressure transducer (Bell and Howell), and blood pressure and heart rate were recorded on
a Devices polygraph. The venous cannula was used for the infusion of drugs. The laser Doppler probe and temperature thermistor were placed on the opposite ear. Insertion of cannulas caused a transient rise in heart rate and blood pressure lasting <5 min in all animals. The rabbits were left undisturbed for ~60 min, until steady baselines were reached.

Four basal measurements were then made at 5-min intervals, after which ANG II (Hypertensin, Ciba) was infused intravenously at incremental doses (0.001, 0.01, and 0.05 µg·kg⁻¹·min⁻¹) for 10 min at each dose. After the last infusion, 15 min were allowed for blood pressure to return to basal levels, after which clonidine hydrochloride (15 µg/kg, Boehringer) was infused intravenously over 10 min. A further 20 min were allowed before ANG II infusions were restarted. Experiments were repeated, on separate days and in random order, in all seven animals, using intracisternal clonidine (1 µg/kg) and intravenous pentolinium tartrate (15 mg/kg, May and Baker) in place of intravenous clonidine. At the end of each experiment, adequacy of sympathetic blockade was tested by the ability of intravenous and intracisternal clonidine and intravenous pentolinium to completely inhibit sympathetic activation induced by intravenous morphine at a dose of 4 mg/kg (22, 23). Animals were lightly restrained through the experiment. Room temperature was maintained at 23 ± 1°C.

Cutaneous ear blood flow and vascular resistance. Continuous measurements of skin blood flow were made using a laser Doppler flowmeter (Periflux model PF2b, Perimed; Ref. 4). The probe was placed on a shaved area, ~1 cm lateral to the central ear artery, on the ventral surface of the ear, using a plastic holder held in place by double-sided adhesive tape. This did not affect baseline measurements. Cutaneous ear vascular resistance was derived from the ratio of blood pressure and cutaneous ear blood flow.

Temperature measurements. Cutaneous temperatures were measured, as additional estimates of cutaneous blood flow, on shaved areas of the ear, adjacent to the laser Doppler probe, and on the back skin, adjacent to the spine (Panlab). Rectal temperature was measured using a rectal temperature probe (Digimed).

Blood collection and analysis. Blood (2.5 ml) was collected from the arterial cannula into cooled tubes containing 50 µl of ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (0.095%)-glutathione (0.06%) for measurement of plasma norepinephrine and epinephrine. The tubes were kept on ice until centrifugation at 4°C, and plasma was stored at −70°C until assay. Catecholamines were separated by high-pressure liquid chromatography and detected electrochemically (21).

Data analysis. Data are presented as means ± SE. Analysis was performed using the SAS statistical programme (SAS Institute, Cary, NC). For the cardiovascular variables, the control value consisted of the mean of the four readings taken during the control period. A logarithmic transformation was performed on the values for norepinephrine and epinephrine before analysis. The data were subjected to analysis of variance, and where the null hypothesis was rejected Schef-fé's comparisons were performed separately on responses to ANG II before and after intravenous clonidine, intracisternal clonidine, and intravenous pentolinium. P < 0.05 was considered significant.

RESULTS

Blood pressure. ANG II caused a dose-related rise in blood pressure in each animal (Fig. 1, Tables 1–3). Blood pressure fell after intravenous clonidine, intracisternal clonidine, and intravenous pentolinium, after which ANG II induced a similar dose-related rise in blood pressure. The increase in blood pressure after each dose of ANG II was not reduced by intravenous clonidine, intracisternal clonidine, or intravenous pentolinium. Intravenous morphine did not induce any further significant changes in mean blood pressure after intravenous clonidine [62 ± 4 to 66 ± 5 mmHg,
Hemodynamic and PNE responses to intravenous ANG II before and after intravenous pentolinium

Table 2. Hemodynamic and PNE responses to intravenous ANG II before and after intravenous pentolinium

<table>
<thead>
<tr>
<th>MAP, mmHg</th>
<th>HR, beats/min</th>
<th>LDF, V</th>
<th>LDR, mmHg/V</th>
<th>PNE, nmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-ANG II</td>
<td>Pre-Pent</td>
<td>Post-Prent</td>
<td>Pre-ANG II</td>
<td>Pre-Pent</td>
</tr>
<tr>
<td>Basal</td>
<td>80 ± 2</td>
<td>64 ± 4†</td>
<td>180 ± 10</td>
<td>185 ± 13†</td>
</tr>
<tr>
<td>0.001 ANG II</td>
<td>80 ± 3</td>
<td>66 ± 3</td>
<td>185 ± 10</td>
<td>178 ± 17</td>
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<td>0.01 ANG II</td>
<td>92 ± 4*</td>
<td>82 ± 3*</td>
<td>195 ± 9</td>
<td>186 ± 17</td>
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<tr>
<td>0.05 ANG II</td>
<td>114 ± 5*</td>
<td>100 ± 5*</td>
<td>195 ± 7</td>
<td>211 ± 22</td>
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<tr>
<td>Post-ANG II</td>
<td>80 ± 7</td>
<td>62 ± 4</td>
<td>218 ± 13</td>
<td>197 ± 9</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7 rabbits. HR, heart rate; LDF, laser-Doppler flow; LDR, laser-Doppler resistance; PNE, plasma norepinephrine; Clon, clonidine. *P < 0.05 compared with basal; †P < 0.05 compared with basal before Clon (post-ANG II).

Table 3. Hemodynamic and PNE responses to intravenous ANG II before and after intravenous pentolinium

<table>
<thead>
<tr>
<th>MAP, mmHg</th>
<th>HR, beats/min</th>
<th>LDF, V</th>
<th>LDR, mmHg/V</th>
<th>PNE, nmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-ANG II</td>
<td>Pre-Pent</td>
<td>Post-Prent</td>
<td>Pre-ANG II</td>
<td>Pre-Pent</td>
</tr>
<tr>
<td>Basal</td>
<td>88 ± 4</td>
<td>67 ± 2†</td>
<td>175 ± 11</td>
<td>206 ± 9</td>
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<tr>
<td>0.001 ANG II</td>
<td>94 ± 6</td>
<td>70 ± 2</td>
<td>183 ± 8</td>
<td>203 ± 10</td>
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<tr>
<td>0.01 ANG II</td>
<td>97 ± 6</td>
<td>73 ± 3</td>
<td>173 ± 8</td>
<td>211 ± 10</td>
</tr>
<tr>
<td>0.05 ANG II</td>
<td>115 ± 8*</td>
<td>86 ± 4*</td>
<td>190 ± 5</td>
<td>215 ± 10</td>
</tr>
<tr>
<td>Post-ANG II</td>
<td>94 ± 4</td>
<td>70 ± 3</td>
<td>186 ± 9</td>
<td>204 ± 8</td>
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</tbody>
</table>

Values are means ± SE; n = 7 rabbits. Pent, pentolinium. *P < 0.05 compared with basal; †P < 0.05 compared with basal before Pent (post-ANG II).

P = not significant (NS), intracisternal clonidine (76 ± 4 to 77 ± 6 mmHg, P = NS), or intravenous pentolinium (70 ± 3 to 69 ± 6 mmHg, P = NS) at end of experiments.

Heart rate ANG II did not induce significant changes in heart rate before or after intravenous clonidine, intracisternal clonidine, or intravenous pentolinium (Tables 1–3).

Cutaneous ear blood flow and vascular resistance. ANG II caused dose-related fall in cutaneous ear blood flow and rise in vascular resistance (Fig. 2, Tables 1–3). Cutaneous ear blood flow increased and vascular resistance fell after intravenous clonidine, intracisternal clonidine, and intravenous pentolinium. ANG II did not lower ear blood flow or raise ear vascular resistance after intravenous clonidine, intracisternal clonidine, or intravenous pentolinium.

Cutaneous temperatures. Before intravenous clonidine, intracisternal clonidine, and intravenous pentolinium, ANG II reduced ear temperature but did not influence back skin or rectal temperature (Table 4). Intravenous clonidine, intracisternal clonidine, and intravenous pentolinium increased ear temperature but not back or rectal temperatures. ANG II did not change temperature at any site after intravenous clonidine, intracisternal clonidine, or intravenous pentolinium.

Plasma norepinephrine and epinephrine. There were no consistent changes in plasma norepinephrine in response to ANG II (Fig. 3; Tables 1–3). Plasma norepinephrine fell significantly in response to ANG II in the first experiment before intravenous clonidine only. Plasma norepinephrine fell after intravenous clonidine, intracisternal clonidine, and after intravenous pentolinium. ANG II lowered plasma norepinephrine after intracisternal clonidine but did not induce any significant changes in norepinephrine after intravenous clonidine or intravenous pentolinium. Plasma epinephrine did not change in response to ANG II before intravenous clonidine (0.16 ± 0.03 to 0.12 ± 0.07 nmol/l, P = NS), intracisternal clonidine (0.13 ± 0.03 to 0.15 ± 0.06 nmol/l, P = NS), or intravenous pentolinium (0.16 ± 0.07 to 0.13 ± 0.04 nmol/l, P = NS). There was a nonsignificant fall in plasma epinephrine after intravenous clonidine, intracisternal clonidine, and intravenous pentolinium. ANG II did not induce any
further changes in epinephrine after intravenous clonidine (0.10 ± 0.06 to 0.09 ± 0.05 nmol/l, P = NS), intracisternal clonidine (0.09 ± 0.02 to 0.12 ± 0.07 nmol/l, P = NS), or intravenous pentolinium (0.08 ± 0.04 to 0.10 ± 0.05 nmol/l, P = NS).

DISCUSSION

Results of the present study suggest that the sympathetic nervous system is not the primary mediator of the acute pressor response to intravenous ANG II and contrast with its constrictor action on cutaneous ear vessels, which is primarily caused by an effect of ANG II on the brain, resulting in activation of the sympathetic nervous system. These results separate, in resting conscious rabbits, the direct vascular and indirect effects of intravenous ANG II.

The physiological mechanisms that mediate the pressor response of ANG II include a direct vasoconstrictor

Fig. 2. Change in laser Doppler resistance (LDR) in response to incremental doses of angiotensin II before and after iv Clon (A), ic Clon (B), and iv Pent (C). *P < 0.05 compared with corresponding values before iv Clon, ic Clon, and iv Pent.

Fig. 3. Percent change in MBP (A), LDR (B), and norepinephrine (C), after iv Clon, ic Clon, and iv Pent compared with basal values.
action, release of vasopressin (2, 11, 12), attenuation of the cardiac baroreflex (10), and activation of the sympathetic nervous system by an action at nerve endings (1, 38), autonomic ganglia (31), and the brain (6, 7, 13, 37). In this study using conscious animals, we have shown that ANG II induced a similar rise in blood pressure, before and after central sympathetic blockade with intravenous and intracisternal clonidine (15, 16, 30), the latter at a dose that is ineffective when given intravenously, and after ganglionic blockade with intravenous pentolinium. Adequacy of sympathetic blockade was confirmed at the end of each experiment by the ability of intravenous and intracisternal clonidine and intravenous pentolinium to abolish the pressor response to intravenous morphine caused by an increase in sympathoadrenal outflow (21–23). Our findings demonstrate that in resting, conscious rabbits the acute pressor response to intravenous infusion of ANG II is predominantly caused by its direct action on blood vessels, and they fail to provide evidence for a significant interaction of ANG II with the sympathetic nervous system. These findings are in accord with those of Cox and Bishop (5), who showed that ganglionic blockade does not abolish the acute, unlike the chronic, pressor effect of ANG II. It should be emphasized that these results apply to rabbits in the resting situation and cannot be extrapolated to animals under heightened sympathetic drive, in which effects of ANG II may be modulated differently by sympathetic activation. A possible role of vasopressin release in the pressor response to ANG II, however, cannot be excluded by the present study.

An interaction of the sympathetic nervous system in the pressor response to ANG II has been suggested by studies reporting attenuation of the pressor response to a bolus injection of ANG II after ganglionic blockade in conscious dogs (9) and after phentolamine in conscious rabbits (34). However, in the study by Fujii and Vatner (9), only transient hemodynamic responses, with peak changes occurring within 1 min, were determined in response to a bolus injection of ANG II. The presence of a bradycardia after bolus ANG II, not generally seen after ANG II infusion or in this study, suggests that hemodynamic responses to ANG II may be influenced by the rate and/or concentration at which ANG II reaches its target sites. Similarly, in the study by Rowe et al. (34), attenuation of the pressor response to ANG II after phentolamine only occurred at the highest (3 µg) bolus dose of ANG II. Paradoxically, the same investigators noted an accentuation of the pressor response to ANG II (3 µg) after ganglionic blockade with hexamethonium. The pressor response to ANG II was unchanged before and after phentolamine or hexamethonium (34), at doses comparable to those used in the present study. Additional evidence supporting involvement of the sympathetic nervous system has come from observations of a higher blood pressure rise after intravertebral or intracerebroventricular ANG II compared with a similar dose administered intravenously (37). This may be caused in part by a specific stimulatory action of ANG II on circumventricular organs such as the area postrema (5, 27) at higher doses. This effect was not contributory in the present study, however, because the ANG II-induced blood pressure rise was similar before and after sympathetic blockade.

Evidence from previous studies suggests that the pressor effect of intravenous ANG II is primarily caused by its vasoconstrictor action on the renal and splanchnic vascular beds, with little effect on the skeletal muscle vasculature (3, 8, 26). These effects are likely to be caused by a direct vascular action of ANG II and to differ from its vasoconstrictor action in the human hand, which is mediated by its indirect effects (17), although the site of this action is not known. Cutaneous blood vessels of the rabbit ear resemble those in the human hand, with an abundance of arteriovenous anastomosis regulated predominantly by sympathetic vasconstrictor nerves (16, 32, 37). In this study, cutaneous vascular responses on the rabbit ear and back were determined to investigate whether the indirect actions of ANG II, mediated by the sympathetic nervous system, could be separated from its direct vascular effects.

<table>
<thead>
<tr>
<th></th>
<th>Ear</th>
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<th>Rectal</th>
<th>Ear</th>
<th>Back</th>
<th>Rectal</th>
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<tbody>
<tr>
<td><strong>Basal</strong></td>
<td></td>
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<tr>
<td>ANG II</td>
<td>33.8 ± 1.7</td>
<td>35.1 ± 0.6</td>
<td>34.0 ± 0.8</td>
<td>34.1 ± 0.1</td>
<td>36.0 ± 0.8</td>
<td>34.1 ± 0.1</td>
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<tr>
<td>Post-ANG II (Basal pre-Clon)</td>
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<tr>
<td>ANG II</td>
<td>35.1 ± 0.6</td>
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<td>36.0 ± 0.8</td>
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<tr>
<td><strong>Post-ANG II (Basal pre-Pent)</strong></td>
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<td>ANG II</td>
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Intravenous ANG II caused dose-related fall in blood flow and cutaneous ear temperature and rise in vascular resistance, indicating vasoconstriction in the cutaneous ear vessels opposite to the site of ANG II infusion. This response is likely to have been mediated centrally, although a possible effect of ANG II on receptors in that ear and, as a result, on the nervous system innervation of the ipsilateral and contralateral ear, cannot be excluded by this study. ANG II did not influence skin temperature, suggesting lack of change in blood flow to this region. However, direct measurements of blood flow to the back, and other regional vascular beds, were not made and remain a limitation of this study. Clonidine and pentalinonium reduced ear vascular resistance, indicating that the vascular tone was maintained by sympathetic mechanisms. The constrictor effect of intravenous ANG II on cutaneous ear vessels was abolished after central sympathetic blockade after Intravenous and intracisternal clonidine and after ganglionic blockade with intravenous pentalinonium, despite the vessel’s potential to constrict in the dilated state after direct administration of ANG II or norepinephrine (18, 35, 36). Our results demonstrate that cutaneous ear vasoconstriction after intravenous ANG II is dependent on its action on the brain, increasing sympathetic nervous activity to the ear, and excludes a direct vascular effect of ANG II on rabbit ear vessels. In contrast, the pressor response to infusion of ANG II depended on its direct vascular actions, and there was no evidence supporting a role for the sympathetic nervous system. As well as being of direct physiological interest, this finding provides a minimally invasive means of investigating the central mechanisms of action of ANG II in conscious animals.

The specific sites at which ANG II acts to cause vasoconstriction in the cutaneous vessels of the ear of the rabbit or in the human hand are unknown. Observations that the effects of intravenous ANG II on hand blood flow are absent in the denervated limbs of patients with unilateral brachial plexus injury who have a postganglionic lesion (17) and in a patient with chronic cervical spinal cord transection with a preganglionic lesion (36) imply that the effect of ANG II on human hand blood flow is dependent on a central action of ANG II, presumably on the circumventricular organs. In the rabbit, angiotensin binding sites are found in the circumventricular organs, in the anterior wall of the third ventricle, and in the area postrema in the brain stem (25). There is evidence from studies with c-fos (24) and studies on the effects of lesions (5, 19) that ANG II acts on both these regions, suggesting that these circumventricular organs could mediate the effect of circulating ANG II on ear blood flow.

Our results demonstrate that the acute pressor response to intravenous ANG II is mediated by its direct vascular effects and is not dependent on the indirect, sympathetically mediated actions of ANG II. The pressor response to intravenous ANG II contrasts with its specific action on cutaneous ear vessels, which is caused entirely by an effect of ANG II in the brain resulting in activation of the sympathetic nervous system. These results separate, in conscious rabbits, the direct vascular effects of ANG II from its indirect sympathetically mediated actions, which induce a selective increase in sympathetic vasconstrictor activity in cutaneous ear vessels.

Address for reprint requests: J. S. Kooner, Cardiology Div., Dept. of Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Rd., London W12 0NN, UK.

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