Investigation of the mechanisms by which chronic infusion of an acutely subpressor dose of angiotensin II induces hypertension

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Running Head: Mechanisms of hypertension induced by low-dose Ang II

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Word count: 5284
Abstract
The mechanisms by which chronic infusion of an initially subpressor low-dose of angiotensin II (Ang II) causes a progressive and sustained hypertension remain unclear. In conscious sheep (n=6), intravenous infusion of Ang II (2μg/h) gradually increased mean arterial pressure (MAP) from 82±3 to 96±5 mmHg over 7 days (P<0.001). This was accompanied by peripheral vasoconstriction, total peripheral conductance decreased from 44.6±6.4 to 38.2±6.7 mL/min/mmHg (P<0.001). Cardiac output and heart rate were unchanged. In the regional circulation, mesenteric, renal and iliac conductances decreased but blood flows were unchanged. There was no coronary vasoconstriction and coronary blood flow increased. Ganglion blockade (hexamethonium, 125 mg/h for 4 hours) reduced MAP by 13±1 mmHg in the control period and by 7±2 mmHg on day 8 of Ang II. Inhibition of central AT-1 receptors by intracerebroventricular infusion of losartan (1 mg/h for 3 hours) had no effect on MAP in the control period or after 7 days Ang II infusion. Pressor responsiveness to incremental doses of intravenous Ang II (5, 10 20 μg/h, each for 15 min) was unchanged after 7 days of Ang II infusion. Ang II caused no sodium or water retention. In summary, hypertension due to infusion of a low dose of Ang II was accompanied by generalized peripheral vasoconstriction. Indirect evidence suggested that the hypertension was not neurogenic, but measurement of sympathetic nerve activity is required to confirm this conclusion. There was no evidence for a role for central angiotensinergic mechanisms, increased pressor responsiveness to Ang II or sodium and fluid retention

Key words: Angiotensin II, ganglion blockade, losartan, regional blood flow, total peripheral resistance
Introduction

The renin angiotensin system plays a central role in the long-term control of arterial pressure, as indicated by its ability to cause sustained hypertension and by the effectiveness of agents that inhibit this system as treatments for hypertension. The hypertension following chronic infusion of angiotensin II (Ang II) has been demonstrated to result from different mechanisms depending on whether a large, immediately pressor dose or a low initially sub-pressor dose is administered (7, 38). It remains unclear how long-term infusion of an initially subpressor dose of Ang II causes a sustained increase in blood pressure that is higher than can be accounted for by the acute vasoconstrictor action of this low dose of Ang II.

Long-term infusion of high doses of angiotensin causes an immediate and maintained increase in blood pressure that is accompanied by increased aldosterone levels, sodium and water retention, and volume expansion (2, 11). Activation of compensatory mechanisms including a resetting of the baroreceptor reflex (6) and inhibition of renal sympathetic nerve activity (3, 9) have been demonstrated. Involvement of neural mechanisms have, however, been suggested by the finding that ganglionic blockade induced a greater fall in blood pressure during chronic compared with acute infusion of angiotensin (23).

In contrast, administration of initially subpressor doses of Ang II causes a gradually developing hypertension, but the peripheral targets for Ang II, the haemodynamic changes and the mechanisms that lead to these changes are unclear. A role for the sympathetic nervous system is supported by studies showing that inhibition of sympathetic activity reduced the pressor response to Ang II (14, 33), although there is evidence that the relative importance of sympathetic activation depends on the level of salt intake (21). Increased sympathetic activity could result from an action of circulating Ang II on circumventricular organs as intra-vertebral or intra-carotid
infusion of low doses of angiotensin II have a greater hypertensive action than intravenous infusion (5, 18, 27). Other possible hypertensive mechanisms include auto-potentiation of the pressor response to Ang II (1, 12) and vascular hypertrophy (15, 22). Although it is widely accepted that the kidneys play an important role in the long-term control of arterial pressure, sodium retention does not appear to be a primary mechanism because low dose Ang II hypertension is accompanied by no (7, 14, 19, 20), or at the most mild (24), sodium retention.

The present experiments examined the responses to chronic infusion of an initially subpressor dose of angiotensin that in the long-term caused hypertension. We believe that this paradigm more closely mimics the development of hypertension in man than the administration of high pressor doses of Ang II. Indeed it has been suggested that the mechanisms by which low doses of Ang II increase blood pressure may be similar to those that cause hypertension due to renal artery stenosis (4, 33). The aim of this study was to examine the effect of infusion of a low, initially subpressor, dose of Ang II that causes a gradual increase in arterial pressure over 7 days in conscious sheep. As the haemodynamic changes over the development of this hypertension have not been studied, we measured systemic and regional haemodynamics. In addition, we investigated (1) the response to ganglion blockade to investigate the role of the sympathetic nervous system, (2) the effect of antagonism of central angiotensin AT-1 receptors to determine the role of central angiotensinergic mechanisms, (3) the vascular reactivity to Ang II to determine whether autopotentiation of the vascular response occurred and (4) sodium and fluid balances to determine whether Ang II caused sodium retention.
Methods

All experiments were conducted on adult Merino ewes (35-45 kg) housed in individual metabolism cages. Six sheep were used for the regional blood flow study, a further 6 sheep to examine the pressor response to Ang II, the effects of ganglion blockade and the response to intracerebroventricular (ICV) infusion of losartan and 8 sheep to determine changes in fluid and electrolyte balances. Sheep were fed a diet of oaten chaff (800 g/day), and water was offered ad libitum. All experiments were approved by the Animal Experimentation Ethics Committee of the Howard Florey Institute.

Surgical procedures

Prior to the studies, sheep for measurement of blood flows underwent three aseptic surgical procedures, each separated by 2 weeks (40). Briefly, animals were anaesthetised with intravenous (IV) sodium thiopental (15 mg/kg) and following intubation, maintained with 1.5-2.0% isoflurane/O₂. Surgeries consisted of i) oophorectomy and bilateral carotid arterial loop placement, ii) implantation of transit-time flow probes (Transonic Systems Inc., Ithaca, NY, USA) on the ascending aorta (20 mm) and the left circumflex coronary artery (3mm), and iii) implantation of transit-time flow probes around the cranial mesenteric (6mm), left renal (4mm), and left external iliac arteries (6mm). Antibiotic (900 mg procaine penicillin, Troy Laboratories, NSW, Australia) was administered prophylactically for 3 days post-surgery. Post-surgical analgesia was maintained with intramuscular injection of flunixin meglumine (1 mg/kg) (Mavlab, Qld, Australia) at the start of surgery, then 4 and 16 hours post-surgery. Experiments commenced at least 2 weeks post-surgery.

For the ICV infusion studies, sheep were prepared with carotid artery loops as described above. After 2 weeks sheep were re-anesthetised, placed in a stereotaxic apparatus and stainless steel
guide tubes were implanted over the lateral cerebral ventricles. Antibiotic treatment and post-
surgical analgesia was as described above.

In all animals, at least one day prior to experiments, using aseptic techniques a Tygon cannula
was inserted into the carotid artery for measurement of arterial pressure. Two further cannulae
were inserted into a jugular vein for measurement of central venous pressure (CVP) and for
infusions.

*Systemic and regional hemodynamic responses to infusion of Ang II*

In six conscious sheep, arterial pressure, central venous pressure, cardiac output (CO) and
regional blood flows were recorded 24 hours/day for a 2 day control period, during 7 days
infusion of Ang II (2μg/h) and during 3 post infusion days. Data from flow probes were collected
via flow-meters (Transonics Systems). A separate circuit measured the first differential of the
upstroke of aortic flow (dF/dt) at each beat from the CO signal. Following analogue to digital
conversion, data were collected on computer at 100 Hz for 10 sec at 5 minute intervals using
custom written software.

*Effects of infusion of Ang II on the pressor responsiveness to Ang II, and the responses to
ganglion blockade and antagonism of central angiotensin AT-1 receptors*

In 6 conscious sheep with ICV guide tubes, arterial pressure was measured at 5 minute intervals
during 6 control days and during 7 days IV infusion of Ang II (2 μg/h). On the morning of the
first control day, progressive doses of Ang II (5, 10 and 20 μg/hr) were infused IV for 15 minutes
at each dose, with data recorded every minute for 15 minutes before, during and 30 minutes after
the dose response and in the afternoon, losartan (1 mg/hr) was infused ICV for 3 hours. On the
second control day hexamethonium (0.5 mg/kg/hr) was infused IV for 4 hours. We have
previously shown that infusion of this dose of losartan for 1 hour inhibits the response to centrally administered Ang II (32) and that the dose of hexamethonium used causes effective ganglion blockade after 2 hours infusion(37). After 2 further control days, during which there was no treatment, an IV infusion of Ang II (2-3μg/hr) was started. The Ang II dose response curves, ICV infusion of losartan and IV infusion of hexamethonium were repeated on days 7 and 8 of Ang II infusion, respectively, with the Ang II infusion being stopped on day 9.

**Fluid and electrolyte balance during infusion of low-dose Ang II**

After 2 control days, Ang II (2 μg/h) was infused intravenously for 7 days followed by 3 post infusion days in 8 sheep. Daily water consumption and urine volume were measured and urine samples were collected for measurement of sodium and potassium concentration using a flame photometer (Technicon, AutoAnalyzer Flame Photometer IV, Canada).

**Statistics**

Cardiovascular variables, grouped into 24 hour means, and fluid and electrolyte data were analysed with one-way repeated measures ANOVA with Bonferroni correction. The increases in MAP during the dose response curves to Ang II, completed before Ang II infusion and on day 7 of Ang II infusion, were compared using two-way repeated measures ANOVA with Bonferroni correction. The effects of losartan and hexamethonium were determined using data from the last 2 hours of the infusions with two-way ANOVA with Bonferroni correction. All analysis was performed using SigmaStat (v. 2.03 Access Softek Inc.). Values are mean ± standard error of the mean (SEM).
Results

Systemic and regional hemodynamic responses to IV infusion of Ang II

Intravenous infusion of Ang II (2 µg/h) had no immediate pressor effect, but caused a gradual, progressive increase in MAP, reaching a plateau after 4 days infusion. MAP was significantly increased by 7.8 ± 1.6 mmHg after 2 days (P=0.002) and by 12.6 ± 2.4 mmHg after 4 days (P<0.001) (Figure 1). Infusion of Ang II had no significant effect on CO, HR or dF/dt, but caused a small increase in stroke volume from Day 1 (+2.3 ± 0.4 mL, P=0.012), reaching a maximum at Day 6 (+4.3 ± 0.4 mL, P<0.001). Total peripheral conductance (TPC) gradually decreased during the infusion of Ang II (-2.2 ± 1.6 mL/min/sec, P=0.007, Day 3), reaching a minimum on day 7 (-7.9 ± 2.0 mL/min/sec, P<0.001).

In the regional vascular beds there were progressive reductions in mesenteric (-1.2 ± 0.3 mL/min/mmHg, P<0.001, Day 7), renal (-0.54 ± 0.10 mL/min/mmHg, P<0.001, Day 7) and iliac (-0.21 ± 0.08 mL/min/mmHg, P=0.014, Day 7) conductances during infusion of Ang II (Figure 2). In contrast, there was no change in coronary conductance throughout the infusion (Figure 2). During Ang II infusion there were no significant changes in mesenteric, renal or iliac blood flows, but coronary blood flow was significantly increased (+5.15 ± 1.55 mL/min, P=0.002, Day 5) (Figure 2).

Following the end of the infusion of Ang II there was a reduction in MAP to control levels after 2 days and to below control by the third post infusion day (Figure 1). Following the end of the infusion HR, CO, TPC, and blood flows and conductances in the mesenteric, renal and iliac vascular beds all tended to increase above control levels by the third post infusion day (Figures 1 and 2).
Pressor responsiveness to Ang II

Intravenous infusion of Ang II at increasing doses during the control period caused stepwise increases in blood pressure, reaching levels significantly above control at infusion rates of 10µg/h (+10 ± 4 mmHg, P=0.015) and 20 µg/h (+15 ± 5 mmHg, P<0.001). After 7 days infusion of Ang II, MAP had increased from 77.2 ± 2.6 mmHg to 91.4 ± 3.9 mmHg (P<0.05, Day 7). An identical dose response to Ang II performed after 7 days IV infusion of Ang II (2 µg/h) caused similar increases in arterial pressure after 10 µg/h (12.6 ± 4.2 mmHg, P=0.039) and 20 µg/h (15.0 ± 3.0 mmHg, P<0.001). The increases in MAP in response to all doses of Ang II (5, 10 and 20 µg/h) given in the control period and after 7 days of low dose IV infusion of Ang II were not significantly different (P=0.953).

Antagonism of central angiotensin AT-1 receptors

During the control period, ICV infusion of losartan (1 mg/h for 3 hours) had no effect on MAP or HR. After 7 days of Ang II infusion, the increase in MAP was not reduced by ICV infusion of losartan and there was no effect on HR.

Ganglion blockade

During the control period, infusion of hexamethonium (0.5mg/kg/hr, 4 hrs) decreased MAP by 12.7 ± 1.4 mmHg (P=0.019). On the 8th day of Ang II infusion, when MAP was increased by 11.8 ± 2.8 mmHg (P=0.03), infusion of hexamethonium decreased MAP by 6.6 ± 4.4 mmHg, but this change was not significant.

Fluid and electrolyte balance during infusion of low-dose AngII

In a separate group of 8 sheep, in which fluid and electrolyte changes were monitored, a slow progressive increase in MAP occurred over 7 days of intravenous Ang II (Figure 4). In these
sheep, there was a gradual increase in urinary sodium excretion that was significant on day 6 of Ang II. There was no initial sodium retention and no natriuresis at the end of the Ang II infusion. Urinary potassium did not show any distinctive trends but was significantly elevated above control on the first and third day of Ang II (Figure 4). Urine volume was increased on days 6 and 7 of Ang II, but there was no significant change in the amount of water drunk.
Discussion

The present study has demonstrated that chronic infusion of an initially subpressor dose of Ang II caused a slowly developing but sustained increase in arterial pressure in sheep. Similar responses to low doses of Ang II have been reported in rats (7), rabbits (13), dogs (4, 11) and humans (2). The main findings were that the hypertension was accompanied by vasoconstriction in the main vascular beds, the hypertension was not dependent on either the sympathetic nervous system, as judged by the response to ganglion blockade, or to central angiotensinergic mechanisms, autopotentiation of the pressor response to Ang II or to sodium retention.

The Ang II-induced hypertension in sheep was accompanied by peripheral vasoconstriction, as shown by the progressive decrease in total peripheral conductance with no change in cardiac output. Similarly in dogs the hypertension due to prolonged infusion of low doses of Ang II was accompanied by peripheral vasoconstriction (10, 33), although in a study in which intermittent measurements of CO were made using dye dilution there was an initial decrease in CO followed by an increase after 4-5 days (10, 33). The progressive increase in arterial pressure in sheep during Ang II was not accompanied by bradycardia, indicating resetting of the baroreflex control of heart rate during infusion of Ang II. This effect of Ang II has been shown to be due to inhibition of cardiac efferent vagal discharge (28) possibly by an action on the area postrema (29), although this is not supported by a recent study (35).

Changes in the regional circulation during the development and maintenance of hypertension due to prolonged low dose Ang II administration have not been studied previously. We found that Ang II infusion for 7 days caused a gradually increasing degree of vasoconstriction in the gut and kidney, with a lesser effect in skeletal muscle. This vasoconstriction prevented any increase in blood flow in these organs in response to the increased perfusion pressure. In contrast, in the
coronary circulation there was no vasoconstriction and coronary blood flow increased, probably in response to the increased cardiac work as coronary blood flow is tightly linked to myocardial oxygen demand. Interestingly, this pattern of response in the regional vasculature differed from that induced by 15 minute infusions of increasing doses of Ang II in conscious sheep, where there was a greater degree of vasoconstriction in the kidney than in the gut and no vasoconstriction in skeletal muscle (31), indicting that different or additional mechanisms are invoked by long-term compared with acute infusion of Ang II.

Stimulation of the sympathetic nervous system has been suggested as a mechanism by which chronic treatment with Ang II causes hypertension. In dogs, it was demonstrated that guanethidine abolished the hypertension caused by Ang II and that the response to tyramine increased as blood pressure increased (33). In rabbits, adrenergic blockade prevented the hypertension caused by 3 days infusion of Ang II, and the importance of the sympathetic nerves was shown by the finding that the pressor action of Ang II was not prevented by adrenalectomy (14). These authors demonstrated that during infusion of a low dose of Ang II the depressor effect of ganglion blockade increased during the first few days of Ang II but this effect decreased after 4 days infusion, suggesting an increasing non-neural component to the hypertension. In rats, hexamethonium induced a greater fall in pressure during chronic low-dose Ang II hypertension compared to control and the chronic component of the hypertension was attenuated by lesion of the subfornical organ and by renal denervation, findings suggesting a neurogenic component to the hypertension (19, 20). In our studies, using low-dose Ang II, the hexamethonium-induced reduction in MAP tended to be lower during infusion of Ang II than during the control period. This finding suggests that the autonomic nervous system does not play a major role in maintaining the hypertension induced by 7 days infusion of Ang II in sheep. There is evidence
that sympathetic activation during low-dose Ang II only occurs in the presence of a high-salt diet (21), which may account for the apparent lack of a neurogenic component to the hypertension because the sheep were fed a normal sodium diet. A limitation of the use of ganglion blockade is that it increases cardiac output (37), probably due to inhibition of vagal tone and the reduction in afterload. This increase in CO during hexamethonium may offset any depressor effects due to inhibition of increased sympathetic activity during Ang II infusion, although there is no reason why this effect should be greater during Ang II than in the control period.

During chronic infusion of an immediately pressor dose of Ang II it has been shown that renal norepinephrine spillover is reduced (9), there is maintained inhibition of renal sympathetic nerve activity (3) and sustained baroreceptor suppression of sympathoexcitatory cells in the rostral ventral lateral medulla (26), suggesting that the sympathetic baroreflex did not reset. Sympathetic nerve activity has not been measured during the hypertension caused by chronic infusion of low doses of Ang II, so it remains to be determined whether in this situation the sympathoexcitatory effect of Ang II is dominant over baroreflex mediated inhibition.

It is well established that the brain angiotensinergic system plays an important role in the central control of blood pressure (34) and it has been demonstrated that chronic infusion of a low dose of Ang II caused a sustained increase in pressure, although this is a sodium dependent hypertension (8, 36). Although Ang II does not cross the blood brain barrier, it is possible that central angiotensinergic mechanisms may play a role in the development of this hypertension, perhaps following stimulation of AT-1 receptors located on the circumventricular organs (16, 19). This hypothesis was not supported, however, by our finding that ICV infusion of losartan for 3 hours, at a dose that inhibited the action of ICV infusion of Ang II (32), did not reduce the blood pressure during the control period or after 7 days treatment with Ang II. We cannot be certain
that losartan infused into the lateral cerebral ventricles reached the relevant AT-1 receptors during the treatment period, although infusion of losartan for 10 or 24 hours in 2 sheep also did not reduce blood pressure during infusion of Ang II (data not shown).

A further mechanism that may account for the peripheral vasoconstriction by this low dose of Ang II is autopotentiation of the pressor response to Ang II (12). The finding in our study that the pressor response to incremental doses of intravenous Ang II was similar during the control period and after 7 days infusion indicates that the vasoconstrictor response to Ang II was not potentiated in this model of Ang II-induced hypertension. Another possibility is that the peripheral vasoconstriction resulted from an action of Ang II on the vasculature to cause wall hypertrophy, as demonstrated in rats after chronic intravenous administration of low-dose Ang II (17, 39). Although the slow return of MAP to normal after termination of the Ang II infusion could be taken as supporting evidence for the development of vascular hypertrophy, the finding that TPC rapidly returned to normal within 24 hours and in fact increased above control, suggests that it is unlikely that significant wall thickening occurred during the 7 days infusion of Ang II.

It is possible that low-dose Ang II hypertension may result from sodium retention due to a direct action on the kidney and by stimulation of aldosterone release. During infusion of Ang II, however, urinary sodium excretion tended increase, suggesting that an increase in plasma volume secondary to sodium retention was not an important factor in the development of the hypertension. This is supported by the finding that there was no initial sodium retention when the Ang II infusion started and no natriuresis at the end of the infusion, in contrast to the large changes seen with high dose Ang II (25) and with of infusion of aldosterone (30). This is in agreement with other studies that have found no changes in fluid or sodium balance during chronic intravenous infusion of Ang II (7, 14, 19, 20).
In conclusion, these studies demonstrate that in sheep chronic infusion of a low dose of Ang II causes an increase in arterial pressure that is greater than can be accounted for by the response to acute infusion of the same dose of Ang II. The sustained hypertension that developed was accompanied by generalised peripheral vasoconstriction without any increase in cardiac output. The mechanisms for this vasoconstriction remain unclear, but are not neurogenic, as judged by the response to ganglion blockade, although direct recordings of sympathetic nerve activity are required to confirm this. In addition, there was no evidence for a role for central angiotensinergic mechanisms, increased pressor responsiveness to Ang II or sodium and fluid retention.
Acknowledgements

The authors are grateful to Craig Thomson and Alan McDonald for excellent technical support.

Grants

This work was supported by an Institute Grant (No. 983001) from the National Health and Medical Research Council of Australia.

Disclosures

None of the authors have any conflicts of interest.
References


Figure Legends

**Figure 1.** Mean arterial pressure (MAP), cardiac output (CO), heart rate (HR) and total peripheral conductance (TPC) in 6 conscious sheep during 2 control days, 7 days intravenous angiotensin II (Ang II) (2 μg/h) and 3 post-infusion days. Results are means±SEM. * P<0.05, † P<0.01, ‡ P<0.001. Significant differences during infusion of Ang II are vs mean of control days.

**Figure 2.** Coronary (CF), mesenteric (MF), renal (RF) and iliac blood flows (IF) (left panels) and conductances (CC, MC, RC, IC) (right panels) in 6 conscious sheep during 2 control days, 7 days intravenous angiotensin II (Ang II) (2 μg/h) and 3 post-infusion days. Results are means±SEM. * P<0.05, † P<0.01, ‡ P<0.001. Significant differences during infusion of Ang II are vs mean of control days.

**Figure 3.** Dose response of mean arterial pressure (MAP) to incremental infusions of Ang II (5, 10, 20 μg/h) before (solid black line) and after 7 days IV infusion of Ang II (2 μg/h)(dashed black line) in 6 sheep. Results are means±SEM. * indicates significant difference from control level within group, there was no significant between the increases in MAP between groups.

**Figure 4.** Mean arterial pressure (MAP) and fluid and electrolyte balance in 8 conscious sheep during 2 control days, 7 days intravenous angiotensin II (Ang II) (2 μg/h) and 3 post-infusion days. Results are means±SEM. * P<0.05, † P<0.01, ‡ P<0.001. Significant differences during infusion of Ang II are vs mean of control days.
Figure 1
Figure 2
Figure 3

MAP (mmHg)

Control  5µg  10µg  20µg

*  *

Control  7 days Ang II
Figure 4