Effects of renal denervation on cardiovascular and renal responses to ACE inhibition in conscious lambs

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Smith, Francine G., Suzanne Chan, and Saskia N. De Wilde. Effects of renal denervation on cardiovascular and renal responses to ACE inhibition in conscious lambs. J. Appl. Physiol. 83(2): 414–419, 1997.—Cardiovascular and renal effects of either the angiotensin-converting enzyme inhibitor captopril or vehicle were measured in chronically instrumented lambs in the presence (intact; n = 6) and absence of renal sympathetic nerves (denervated; n = 5) to determine whether there was an interaction between the renin-angiotensin system and renal sympathetic nerves early in life. Captopril caused a similar decrease in mean arterial pressure (P < 0.001) in intact and denervated lambs, predominantly through a decrease in diastolic pressure. Heart rate was increased from 177 ± 34 to 213 ± 22 (SD) beats/min during captopril compared with vehicle infusion in intact lambs. In denervated lambs, basal heart rates were elevated to 218 ± 33 beats/min; there was no further increase in heart rate during captopril compared with vehicle infusion. Captopril infusion caused a decrease in renal vascular resistance but only in the absence of renal nerves. These findings provide evidence to suggest that early in life there is an interaction between renal sympathetic nerves and the renin-angiotensin system in regulating renal hemodynamics and the baroreflex control of the heart.

angiotensin-converting enzyme; renal nerves; newborn; angiotensin II; captopril; blood pressure; baroreflex

FROM STUDIES IN ADULT animals and humans, it is now generally agreed that there is an important interaction between the renin-angiotensin and sympathetic nervous systems (16, 21) in influencing cardiovascular and fluid and electrolyte homeostasis. In addition to its effects on the peripheral vasculature and on the kidney, angiotensin II exerts several actions on the sympathetic nervous system including central effects on sympathetic outflow, stimulatory effects on the adrenal medulla and sympathetic ganglia, and facilitatory effects on neural transmission at sympathetic nerve terminals. These effects of angiotensin II on the sympathetic nervous system appear to be more pronounced when the renin-angiotensin system is activated, such as occurs under disease conditions like heart failure.

In the newborn period, the renin-angiotensin system is also activated (11), although the reasons for this are not known. It is now well recognized, however, that removal of the effects of angiotensin II before birth by maternal administration of angiotensin-converting enzyme (ACE) inhibitors such as captopril causes fetal and/or neonatal (2, 9, 15, 19) demise including middle-to-late trimester onset of oligohydramnios and intrauterine growth restriction, fetal and/or neonatal hypotension and anuria, and pulmonary hypoplasia, resulting in neonatal death usually in the first week of postnatal life (9, 15). The mechanism of fetal and/or neonatal demise associated with deleterious effects of ACE inhibitors administered before birth remains unknown.

On the basis of the facts that the renin-angiotensin system is elevated early in life and the greater role of the sympathetic system in influencing cardiovascular homeostasis when the renin-angiotensin system is activated, we proposed that there may be an important interaction between the renin-angiotensin and sympathetic systems in influencing cardiovascular homeostasis in the newborn period. If this were the case, then the cardiovascular and renal effects of an ACE inhibitor would be altered in the absence of renal sympathetic nerves.

Experiments were therefore carried out to determine the effects of the ACE inhibitor captopril on various parameters of cardiovascular and renal function in conscious, chronically instrumented lambs and to ascertain whether these effects of captopril were greater in the absence of renal sympathetic nerves. These data may provide important insight into the role of angiotensin II in regulating cardiovascular and renal function early in life, as well as any interaction between the renin-angiotensin system and renal sympathetic nerves in regulating systemic and/or renal hemodynamics in the newborn period.

METHODS

Experiments were performed at least 4 days after surgery in conscious, chronically instrumented lambs with either bilateral renal denervation performed at the time of surgery (denervated; n = 5) or sham denervation with renal nerves remaining intact (intact; n = 6). Postnatal ages and body weights at the time of experiments were as follows: denervated: 13 ± 3 days, 7.5 ± 1.2 kg; intact: 17 ± 4 days, 7.9 ± 1 kg. Lambs were obtained from a local source (Treco Ranch) and housed with their mothers in individual pens in the vivarium, except during surgery and experiments. All surgical and experimental procedures were carried out in accordance with the “Guide to the Care and Use of Experimental Animals” provided by the Canadian Council on Animal Care and with the approval of the Animal Care Committee of the University of Calgary.

Surgical procedures. Surgery was performed on newborn lambs 2–5 days after birth by using aseptic techniques, halothane anesthesia, and techniques previously detailed (23, 25). Femoral vessels were catheterized (PE-160 catheter, Intramedic) for later intravenous infusion and arterial sampling. By means of an abdominal midline incision the bladder was then exposed, and a catheter was inserted directly across the bladder wall. Catheters were tunneled subcutaneously to exit the lamb on the right and left flanks. The left renal artery was approached, and a precalibrated ultrasonic flow transducer (35, 45, Transonics Systems) was placed around the left
renal artery for later measurement of renal blood flow (RBF), as previously described (6).

In the lambs submitted to bilateral renal denervation, the right kidney was located by using a right-flank incision; renal nerves were located, severed, and stripped from along the aorta, renal arteries, and veins as previously described, followed by careful application of 10% phenol in absolute alcohol. The same procedure was repeated for the left kidney. This bilateral renal denervation procedure has previously been shown to reduce renal norepinephrine content by ~97% (24). Sham-operated lambs were submitted to the same surgical procedure, except that renal nerves were left intact and no phenol was applied. Flank incisions were then closed, and the lamb was allowed to recover from the effects of surgery and anesthesia in a critical care unit for small animals (Shor-line, Schroer Manufacturing) with adjustable oxygen supply.

Catheters were contained in pouches on a lamb body jacket (Lomir). Antibiotics (0.5 mg/kg enrofloxacin, Baytril) were administered intramuscularly at surgery and at 12-h intervals thereafter for 48 h. All lambs were able to stand within 60 min of completion of surgery, at which point they were returned to the vivarium.

Experimental details. Two experiments [captopril infusion (experiment 1); vehicle infusion (experiment 2)] were carried out in each lamb at intervals of 2–5 days; the order of experiments was randomized. On the day of an experiment the lamb was removed from the vivarium and placed in a supportive sling in the laboratory environment for at least 60 min.

During this time the bladder was allowed to drain. A priming dose of [14C]inulin (0.5 µCi/kg iv) in dextrose was injected, followed by constant infusion at 0.25 µCi·kg⁻¹·h⁻¹ (0.5 ml·kg⁻¹·h⁻¹ iv) for later measurement of glomerular filtration rate (GFR). An infusion of captopril (0.12 mg·kg⁻¹·h⁻¹ iv, Sigma Chemical) in vehicle (5% dextrose, 0.9% sodium chloride, 4.17 ml·kg⁻¹·h⁻¹) (experiment 1) or of vehicle alone (experiment 2) was started and continued for the duration of the study. The dose of captopril was selected from dose-response curves established in our laboratory as the lowest dose at which the pressor response to 0.5 µg/kg of angiotensin I was 100% inhibited. The dose of angiotensin I (0.5 µg/kg) was tested before and 15 min after the start of intravenous infusion of captopril and at the end of the experiment. In all instances, the pressor response to angiotensin I was 100% inhibited in the presence of captopril infusion.

After the 60-min equilibration period, the experiment was started. Each experiment consisted of consecutive 20-min urinary collection periods for 3 h (9 × 20 min). At the end of each 20-min collection period, urinary volume was recorded, and samples were stored at −70°C for later determination of urinary electrolytes (Na⁺, K⁺) and urinary osmolality. At the midpoint of each 20-min collection period, 2 ml of arterial blood was removed and centrifuged; supernatant was removed and stored at −70°C for later determination of plasma electrolytes (Na⁺, K⁺) and plasma osmolality.

The arterial catheter, advanced to the abdominal aorta, was connected to a pressure transducer (Statham P23Db) for measurement of arterial pressure; the flow transducer was connected to a flowmeter (T101, Transonics Systems) for measurement of RBF. Blood pressures and RBF were recorded onto a polygraph (model 7, Grass Instruments) and simultaneously to an IBM 486 personal computer at 200 Hz by using the data-acquisition and -analysis software package CVSOFT (Odessa Systems).

At the end of the two experiments performed in random order at intervals of 2–5 days, the lambs were killed with a lethal dose of pentobarbital sodium. Catheter placement was verified by postmortem inspection, and the zero offset of the flow transducer was determined.

Analytic procedures. Urinary and plasma [14C]inulin levels were determined immediately after each experiment by using a liquid scintillation counter (Wallace 1410). Urine and plasma samples were later thawed to room temperature, and urinary and plasma electrolytes (Na⁺, K⁺) and osmolalities were measured by using a flame photometer (IL-943) and microosmometer (model 3MO, Advanced Instruments), respectively.

Computations. GFR was calculated as the clearance of [14C]inulin. Systolic, diastolic, and mean blood pressures, RBF, and renal vascular resistance (RVR) were calculated, and heart rates were determined from the systolic peak of the pulsatile waveform by using CVSOFT; additional calculations were performed by using Lotus 1–2–3.

Statistical analyses. For statistical analysis, three-way analysis of variance procedures for repeated measures were applied by using SPSS-PC to determine whether drug (vehicle or captopril), treatment (intact or denervated), or time (0–180 min) had any effects on the measured variables. Significance was accepted at the 95% confidence interval. Where the F-value was found to be significant, (i.e., P < 0.05), Newman-Keuls tests were applied to determine where the significant differences occurred.

Because it was determined from the above statistical tests that time had no effects on the measured variables, data collected over the 3-h experiment were pooled for presentation in Figures 1-3 and Tables 1-2 as means ± SD.

RESULTS

Effects of captopril in intact lambs. Captopril caused a decrease in mean blood pressure (P < 0.001) (Fig. 1) through a decrease in diastolic blood pressure (vehicle: 59 ± 7 mmHg; captopril: 45 ± 9 mmHg) (P < 0.001) and systolic blood pressure (P = 0.04) (vehicle: 100 ± 9 mmHg; captopril: 87 ± 13 mmHg). There was also a decrease in RBF during captopril infusion compared with vehicle infusion (P < 0.0) (Fig. 2) so that RVR remained constant during captopril infusion (0.96 ± 0.1 mmHg·ml⁻¹·min⁻¹) compared with vehicle infusion (1.02 ± 0.4 mmHg·ml⁻¹·min⁻¹). Heart rate increased by >30 beats/min during captopril infusion compared with vehicle infusion (Fig. 3).

![Fig. 1. Mean arterial pressure (MAP) measured during intravenous infusion of either vehicle (open bars) or captopril (solid bars) in lambs with intact renal sympathetic nerves (Intact) or with bilateral renal denervation (Denervated). Values are means ± SD. *P < 0.05 compared with vehicle infusion.](http://jap.physiology.org/10.1152/japplphys.00333.2017)
In intact lambs, GFR was significantly reduced ($P \leq 0.05$) during captopril compared with vehicle infusion (Table 1). Urinary flow, $Na^+$ and $K^+$ excretion rates, and urinary osmolalities remained constant during captopril and vehicle infusion (Table 1). Plasma $Na^+$ and $K^+$ levels and plasma osmolalities also remained constant during captopril and during vehicle infusion (Table 2).

Effects of denervation. Renal denervation significantly increased basal heart rate as illustrated in Fig. 3, confirming our previous findings (25). Plasma osmolality was also decreased in denervated lambs compared with intact lambs ($P = 0.04$; Table 2). Other measured variables were not significantly altered by renal denervation.

Effects of captopril in denervated lambs. The decrease in mean arterial pressure ($P < 0.001$) during captopril infusion in lambs with bilateral renal denervation was similar to that seen in intact lambs (Fig. 1). This decrease in mean arterial pressure was due to a decrease in diastolic pressure (vehicle: $54 \pm 7$ mmHg; captopril: $42 \pm 5$ mmHg) as well as systolic pressure (vehicle: $95 \pm 12$ mmHg; captopril: $87 \pm 5$ mmHg). RBF was not decreased during captopril infusion ($76.5 \pm 7.5$ ml/min) compared with vehicle infusion ($74.8 \pm 7.3$ ml/min) (Fig. 2). In contrast to the lack of change in RVR in intact lambs, captopril infusion decreased RVR ($0.81 \pm 0.07$ mmHg·ml⁻¹·min⁻¹) compared with vehicle infusion ($1.02 \pm 0.14$ mmHg·ml⁻¹·min⁻¹). Heart rate remained constant during captopril infusion in denervated lambs (Fig. 3) compared with the increase in heart rate seen in lambs with intact renal nerves.

GFR was significantly reduced by captopril in denervated lambs, as in intact lambs (Table 1); this response was similar in both groups. Urinary flow ($P = 0.50$), $Na^+$ excretion ($P = 0.77$), and $K^+$ excretion ($P = 0.09$) remained constant during captopril infusion in denervated lambs (Table 1). Plasma electrolytes and plasma osmolality also remained constant during captopril infusion (Table 2) compared with vehicle infusion in denervated lambs.

### DISCUSSION

The data described herein provide new information on the interaction between the renin-angiotensin and sympathetic systems in the newborn. Novel findings of the present study in conscious lambs are that the ACE inhibitor captopril 1) causes a decrease in systolic and diastolic pressures in the presence and absence of renal sympathetic nerves; 2) causes an increase in heart rate in lambs with intact renal nerves; 3) causes a decrease

### Table 1. Effects of vehicle or captopril on renal function in intact and denervated lambs

<table>
<thead>
<tr>
<th></th>
<th>Intact Lambs (n = 6)</th>
<th>Denervated Lambs (n = 5)</th>
</tr>
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<tbody>
<tr>
<td>Glomerular filtration rate, ml/min</td>
<td>22 ± 12</td>
<td>25 ± 7</td>
</tr>
<tr>
<td>Captopril</td>
<td>17 ± 2*</td>
<td>17 ± 9*</td>
</tr>
<tr>
<td>Urinary flow rate, ml/min</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>Captopril</td>
<td>0.2 ± 0.1</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>Urinary $Na^+$ excretion rate, µmol/min</td>
<td>1.7 ± 0.8</td>
<td>3.2 ± 2.3</td>
</tr>
<tr>
<td>Vehicle</td>
<td>2.1 ± 1.6</td>
<td>2.3 ± 1.5</td>
</tr>
<tr>
<td>Captopril</td>
<td>15 ± 11</td>
<td>12 ± 8</td>
</tr>
<tr>
<td>Urinary $K^+$ excretion rate, µmol/min</td>
<td>14 ± 8</td>
<td>13 ± 11</td>
</tr>
<tr>
<td>Vehicle</td>
<td>538 ± 320</td>
<td>607 ± 291†</td>
</tr>
<tr>
<td>Captopril</td>
<td>564 ± 228</td>
<td>763 ± 145†</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of animals. Intact lambs, lambs with intact renal sympathetic nerves; denervated lambs, lambs with bilateral renal denervation. *$P < 0.05$ compared with vehicle infusion. †$P < 0.05$ compared with intact lambs.

### Table 2. Effects of vehicle or captopril on plasma variables

<table>
<thead>
<tr>
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<th>Intact Lambs (n = 6)</th>
<th>Denervated Lambs (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma $Na^+$, mmol/l</td>
<td>142 ± 2</td>
<td>140 ± 1</td>
</tr>
<tr>
<td>Captopril</td>
<td>142 ± 3</td>
<td>140 ± 1</td>
</tr>
<tr>
<td>Plasma $K^+$, mmol/l</td>
<td>3.6 ± 0.1</td>
<td>3.7 ± 0.3</td>
</tr>
<tr>
<td>Captopril</td>
<td>3.7 ± 0.4</td>
<td>4.0 ± 0.4</td>
</tr>
<tr>
<td>Plasma osmolality, mosmol/kgH2O</td>
<td>295 ± 7</td>
<td>290 ± 8†</td>
</tr>
<tr>
<td>Vehicle</td>
<td>298 ± 8</td>
<td>289 ± 6†</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of animals. *$P < 0.05$ compared with vehicle infusion. †$P < 0.05$ compared with intact lambs.
in RVR but only in the absence of renal nerves; and 4) causes a decrease in GFR regardless of the state of renal innervation. Therefore, our hypothesis is that the effects of ACE inhibition would be greater in denervated lambs holds true for renal hemodynamics but not for renal function. Our observations provide new evidence to support the hypothesis that there is an interaction between the renal sympathetic nervous system and the renin-angiotensin system in modulating renal hemodynamics as well as the baroreflex control of heart rate early in life.

The fact that ACE inhibitors like captopril decrease blood pressure has been well documented, at least in adults, and forms the basis for the use of this group of drugs. ACE inhibitors, in the treatment of various hypertensive disorders. We also observed a decrease in blood pressure during ACE inhibition in conscious lambs, presumably as the result of removal of the effects of angiotensin II on peripheral vascular resistance; this was not altered by renal denervation. In addition, captopril caused an increase in heart rate, which was likely mediated by activation of arterial and/or cardiopulmonary baroreflexes in response to the decrease in blood pressure after removal of the effects of circulating angiotensin II. In adult animals, angiotensin II modulates the vagal control of the heart as well as central regulation of sympathetic outflow (5, 8, 10). Resetting of the arterial baroreflex control of heart rate in adult animals is mediated by angiotensin II receptor subtype AT1, and basal angiotensin II is known to exert a tonic action on the set point around which the cardiac baroreflex operates (29). In newborn animals, however, the role of angiotensin II in regulating the baroreflex control of the heart is less well understood. In a recent 9-h study in paralyzed and artificially ventilated lambs, Segar et al. (22) observed a decrease in heart rate and renal sympathetic nerve activity after enalapril administration (22); blood pressures were, however, maintained at the pre-enalapril level by infusion of the pressor agent phenylephrine. In a previous study by the same group (18), the ACE inhibitor captopril decreased blood pressure but did not alter heart rate in conscious lambs. This apparent discrepancy with our present observations in conscious lambs may be related to the wide age range of animals they studied (8–21 days) as reflected in their large variability in control heart rates (SD = 45 beats/min). The baroreflex-mediated increase in heart rate that we observed during ACE inhibition confirms our previous findings (6) in conscious lambs.

In lambs with bilateral renal denervation, this increase in heart rate did not occur. In denervated lambs, however, basal heart rates were already elevated, as has been previously observed (25), suggesting that renal afferents may play a physiological role in providing input from the kidneys to other structures, such as central pathways involved in baroreflex control of the heart.

Neural pathways by which reflexes of renal origin are elicited in adult animals have been investigated by using either tracer techniques or direct renal nerve stimulation. It is well established that renal afferents enter the spinal cord in the lower thoracic and upper lumbar segments and either extend to the dorsal columns where they terminate in the medulla close to the nucleus of the solitary tract or enter Lissau's tract and travel medially in spinoreticular or spinothalamic tracts that terminate in the medulla oblongata and pons or in the thalamus. There is considerable evidence that renal afferents are intimately involved in the reflex regulation of the cardiovascular system including blood pressure, heart rate, central sympathetic outflow, and release of arginine vasopressin (20, 27, 28).

Any functional role of renal afferents early in life has not been investigated nor have these ascending pathways been explored.

The lack of an increase in heart rate in denervated lambs after infusion of captopril was surprising. In a recent study investigating the effects of renal denervation on the cardiovascular responses to acute blood volume depletion with furosemide (25), an increase in heart rate was observed after furosemide administration to conscious lambs, suggesting that it is possible to induce a further increase in heart rate, even in the absence of renal nerves. In that study, however, there was no decrease in blood pressure, suggesting that mechanisms other than the arterial baroreflex were responsible for the heart rate response. It is therefore possible that despite recruitment of baroreflex pathways after the decrease in blood pressure that occurred with ACE inhibition, heart rates cannot be increased further due to their already high basal levels. This warrants further investigation.

An alternative explanation for the lack of increase in heart rate during ACE inhibition in conscious lambs is that there is an interaction between the sympathetic nervous system and the renin-angiotensin system in the control of the heart early in life, perhaps through the action of renal afferents on the central pathways involved in the baroreflex control of the heart and/or sympathetic outflow. In a previous study (26), it was also demonstrated that there is an increase in plasma aldosterone levels after renal denervation in conscious lambs, which is in agreement with the idea that removal of the sympathetic system causes activation of compensatory mechanisms such as the renin-angiotensin-aldosterone system. In the present study in response to captopril infusion we saw no increase in heart rate in denervated lambs, whereas there was no difference in the blood pressure response to captopril in intact compared with denervated lambs. Therefore, renal afferents may play a different role in regulating heart rate in the newborn period than later in life, as has been previously suggested (25).

A renal vasodilation, an increase in ultrafiltration, and an increase in electrolyte excretion during ACE inhibition have been well documented in adult animals (3, 13, 14); these responses are greater when the renin-angiotensin system is activated by prior sodium restriction. Because renin and angiotensin II levels are high in the weeks after birth (11), we predicted that...
there would also be a significant increase in GFR and electrolyte excretion during ACE inhibition in conscious lambs. On the contrary, we observed a decrease in GFR in the intact animal, which probably reflects a decrease in renal perfusion in the absence of changes in pre- and/or postglomerular resistance. This may result from maturational differences in the effects of angiotensin II on the glomerular capillaries, including differential effects on the afferent and/or efferent arterioles. To date, no studies have been carried out to investigate the role of angiotensin II in influencing pre- and/or postglomerular resistance vessels during fetal and newborn life. We observed a renal vasodilation in the presence of ACE inhibition but only when renal nerves were absent. The mechanism(s) governing this response was not determined in the present study. Interestingly, our data suggest that there is a dissociation between the effects of captopril on renal hemodynamics and glomerular filtration. This phenomenon can be explained if GFR and RBF are not autoregulated in newborn lambs over the range of pressures seen in this study. To date, it is not known whether the newborn kidney can exhibit any autoregulatory capacity. This interesting observation warrants further investigation.

ACE inhibitors like captopril are effective inhibitors of converting enzyme or kininase II, which prevent not only the conversion of angiotensin I to angiotensin II but also the degradation of bradykinin (12), leading to a potentiation of the systemic and renal effects of kinins. Therefore, it is possible that some of the effects of ACE inhibition were mediated by increased levels of circulating kinins. Although it is known from in vitro studies in rats that the individual components of the renal kinin-kinin system are developmentally regulated (7), the cardiovascular and renal effects of kinins during postnatal maturation are not yet known.

With the recent characterization of two distinct angiotensin II receptor subtypes (AT₁ and AT₂), a differential distribution of AT₁ and AT₂ receptors has been observed in fetal and newborn kidneys, compared with the distribution seen in the adult kidney (1, 17), with high levels of AT₂ binding predominating in cortical and medullary regions of the human fetal kidney (4) as well as in developing glomeruli of newborn rats (1), the binding decreasing with maturation. The physiological role of angiotensin II receptors in the newborn kidney remains to be elucidated.

Conclusion and prospective. These data provide evidence to suggest that intrarenal and/or circulating angiotensin II is necessary for maintenance of normal blood pressure, ultrafiltration, and RBF during the first few weeks of life. Our observations of a significant decrease in GFR and RBF during ACE inhibition in conscious lambs may also provide some insight into the deleterious effects on the fetus and newborn of agents such as captopril after administration to the pregnant woman. Our present observations of differential heart rate and renal hemodynamic responses to captopril in the presence and absence of renal nerves suggest that early in life there is an interaction between renal sympathetic nerves and the renin-angiotensin system in influencing renal hemodynamics as well as the baroreflex control of the heart.

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