Renal denervation alters cardiovascular and endocrine responses to hemorrhage in conscious newborn lambs

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Smith, Francine G., and Isam Abu-Amarah. Renal denervation alters cardiovascular and endocrine responses to hemorrhage in conscious newborn lambs. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H285–H291, 1998.—To investigate the role of renal sympathetic nerves in modulating cardiovascular and endocrine responses to hemorrhage early in life, we carried out three experiments in conscious, chronically instrumented lambs with intact renal nerves (intact; n = 8) and with bilateral renal denervation (denervated; n = 5). Measurements were made 1 h before and 1 h after 0, 10, and 20% hemorrhage. Blood pressure decreased transiently after 20% hemorrhage in intact lambs and returned to control levels. In denervated lambs, however, blood pressure remained decreased after 60 min. After 20% hemorrhage, heart rate increased from 170 ± 16 to 207 ± 18 beats/min in intact lambs but not in denervated lambs, in which basal heart rates were already elevated to 202 ± 21 beats/min. Despite an elevated plasma renin activity (PRA) measured in denervated (12.0 ± 6.4 ng ANG I·ml⁻¹·h⁻¹) compared with intact lambs (4.0 ± 1.1 ng ANG I·ml⁻¹·h⁻¹), the increase in PRA in response to 20% hemorrhage was similar in both groups. Plasma levels of arginine vasopressin increased from 11 ± 8 to 197 ± 246 pg/ml after 20% hemorrhage in intact lambs but remained unaltered in denervated lambs from baseline levels of 15 ± 10 pg/ml. These observations provide evidence that in the newborn, renal sympathetic nerves modulate cardiovascular and endocrine responses to hemorrhage.

Arginine vasopressin; plasma renin activity; blood pressure; heart rate; renal blood flow; renal vascular resistance; neonate; blood loss; blood volume;

The renal sympathetic nervous system is intimately involved in modulating fluid and electrolyte homeostasis, through its effects on renin release, renal vascular resistance, and glomerular and tubular function (13). A decrease in vascular volume such as occurs after hemorrhage is associated with activation of renal sympathetic nerves (15, 18), which promotes fluid and electrolyte reabsorption as well as the release of the enzyme renin, thereby leading to increased circulating levels of the pressor agent angiotensin II.

Activation of the renin-angiotensin system after increased renal sympathetic activity (9, 16, 22, 23) plays an important role in the physiological responses to hemorrhage, at least in the adult. Furthermore, increased levels of angiotensin II in response to hemorrhage are necessary for blood pressure recovery, because the return of blood pressure towards normal is impaired in the presence of the angiotensin II antagonist saralasin (10) and the angiotensin-converting enzyme inhibitor captopril (20, 42).

In recent studies, we have investigated some of the physiological responses to hemorrhage in conscious lambs in the first week of life (see Ref. 34). Our data provide evidence that at least some of the physiological responses to hemorrhage appear to be developmentally regulated. For example, we observed that in response to hemorrhage of up to 20% of vascular volume, blood pressure decreases only transiently and is restored to prehemorrhage levels very rapidly. These data provide evidence to suggest that there may be a rapid activation of the sympathetic nervous system and, subsequently, activation of the renin-angiotensin system in response to blood loss early in life. To date, however, the mechanisms governing the physiological responses to hemorrhage early in life are poorly understood.

The purpose of the present study was, therefore, to determine the role of renal sympathetic nerves in modulating the cardiovascular and endocrine responses to hemorrhage in conscious newborn lambs. To this end, we measured some of the cardiovascular and endocrine responses to hemorrhage of 0, 10, and 20% of vascular volume in conscious, chronically instrumented newborn lambs with either intact renal sympathetic nerves (intact) or bilateral renal denervation (denervated).

METHODS

Experiments were performed at least 2–5 days after surgery in 13 conscious, chronically instrumented lambs with intact renal nerves (n = 8; intact) or with bilateral denervation (n = 5; denervated). Ages and body weights at the time of experiments are shown in Table 1 for both groups of lambs. Animals were obtained from a local source (Sheep Advisory Service) and housed with their mothers in individual pens in the vivarium of the Health Sciences Centre 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 at least 2 days after birth using aseptic techniques as previously described (12, 33, 38). Briefly, anesthesia was induced with a mask and halothane (3–4%) in oxygen, the trachea was intubated, and anesthesia was maintained by ventilating the lungs with halothane (0.5–1%) in a mixture of nitrous oxide and oxygen (3:1, vol/vol).

Femoral vessels were catheterized (PE-160 catheter, Intramedic) for later intravenous infusions and blood volume withdrawal (right and left femoral veins) and blood pressure recording (right femoral artery). Catheters were tunneled subcutaneously to exit the lamb on the right and left flanks.

Denervated lambs were then submitted to bilateral renal denervation as follows. By means of a right flank incision, renal nerves were located, severed, and stripped from along the aorta, renal arteries, and veins as previously described (36–38). This was followed by careful application of 10%...
Experimental details. Three experiments were carried out in each lamb at intervals of 2–4 days: experiment 1, 0% hemorrhage; experiment 2, 10% hemorrhage; experiment 3, 20% hemorrhage. 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. An intravenous infusion of 5% dextrose in 0.9% sodium chloride (4.17 ml·kg⁻¹·h⁻¹) was started and was continued for the duration of the study. The arterial catheter, advanced to the abdominal aorta, was connected to a pressure transducer (Statham, P23 Db) for measurement of arterial pressure; the flow transducer was connected to a flowmeter (T101, Transonics Systems) for measurement of renal blood flow. These variables were recorded continuously onto a polygraph (model 7, Grass Instruments) and simultaneously to a 486 IBM PC at 200 Hz using the data acquisition and analysis software package CVSOFT (Odessa Systems).

After the 60-min equilibration period, the experiment was started. Each experiment consisted of control measurements (60 min), followed by blood volume depletion of 0, 10, or 20% over 10 min using a programmable syringe pump (model 55–4143, Harvard Apparatus) and the methods previously detailed by us (34). Measurements were continued during and after hemorrhage (60 min). At the end of each experiment, blood withdrawn during hemorrhage was transfused back into the animal over 10 min before the animal was returned to its home cage, where it was housed with the ewe. Blood was removed during the control period and at 20 and 60 min after hemorrhage for later measurement of plasma renin activity and plasma levels of arginine vasopressin. For determination of plasma renin activity, 4 ml of blood were placed in chilled Vacutainer tubes [EDTA(K3), Becton-Dickinson]; 4 ml of blood were also placed in chilled 5-ml polypropylene tubes containing heparin (10 U/ml, HepaLean, Organon Technika) for later measurement of plasma levels of arginine vasopressin. Whole blood was centrifuged at 4°C, and supernatant was removed and stored immediately at −70°C. Blood samples were replaced immediately with previously obtained maternal blood to avoid any effects of sampling. Plasma renin activity and plasma levels of arginine vasopressin were later measured on samples thawed to room temperature using standard radioimmunoassay techniques (17, 32, 34).

At the end of the three experiments performed in random order at intervals of 2–4 days, lambs were killed with a lethal dose of pentobarbital sodium. Catheter placement was verified by postmortem inspection, and the zero offset of the renal blood flow transducer was determined.

Computations. Mean blood pressures, renal blood flow, and renal vascular resistance were calculated and heart rates were determined from the systolic peak of the pressure waveform using CVSOFT. Cardiovascular data were averaged over consecutive 20-min intervals using CVSOFT and a spreadsheet (Microsoft Excel, version 7.0).

Statistical analyses. For statistical analyses, ANOVA procedures for repeated measures were applied to determine whether hemorrhage (0, 10, or 20%), treatment (intact or denervated), or time (control to 60 min) altered the measured variables. Where the F value was found to be significant, Newman-Keuls tests were applied to determine where the significant difference(s) occurred. All data are expressed as means ± SD.

Table 1. Baseline measurements in intact and denervated lambs

<table>
<thead>
<tr>
<th></th>
<th>Intact (n = 6)</th>
<th>Denervated (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, days</td>
<td>6–11</td>
<td>6–11</td>
</tr>
<tr>
<td>Body wt, kg</td>
<td>8±3</td>
<td>8±4</td>
</tr>
<tr>
<td>Blood pressure, mmHg</td>
<td>81±7</td>
<td>91±10*</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>170±16</td>
<td>202±21*</td>
</tr>
<tr>
<td>Renal blood flow, ml/min</td>
<td>83±12</td>
<td>80±24</td>
</tr>
<tr>
<td>Renal vascular resistance, mmHg·ml⁻¹·min⁻¹</td>
<td>1.02±0.12</td>
<td>1.34±0.44</td>
</tr>
<tr>
<td>Plasma renin activity, ng·ANG I·ml⁻¹·min⁻¹</td>
<td>4.0±1.1</td>
<td>12.0±6.4*</td>
</tr>
<tr>
<td>Plasma arginine vasopressin, pg/ml</td>
<td>11±8</td>
<td>15±10</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = no. of animals. *P < 0.05 compared with intact lambs.

Table 2. Renal hemodynamic effects of hemorrhage in intact and denervated lambs

<table>
<thead>
<tr>
<th>Percent Hemorrhage</th>
<th>Control</th>
<th>20 min</th>
<th>40 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBF, ml/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td>83±12</td>
<td>81±11</td>
<td>82±11</td>
<td>83±16</td>
</tr>
<tr>
<td>Denervated</td>
<td>80±24</td>
<td>82±23</td>
<td>88±28</td>
<td>83±22</td>
</tr>
<tr>
<td>10%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td>84±8</td>
<td>92±13</td>
<td>86±12</td>
<td>84±12</td>
</tr>
<tr>
<td>Denervated</td>
<td>88±27</td>
<td>89±27</td>
<td>91±28</td>
<td>92±27</td>
</tr>
<tr>
<td>20%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td>93±14</td>
<td>86±24</td>
<td>83±12</td>
<td>81±14</td>
</tr>
<tr>
<td>Denervated</td>
<td>87±24</td>
<td>78±18</td>
<td>79±25</td>
<td>80±24</td>
</tr>
<tr>
<td>RVR, mmHg·ml⁻¹·min⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td>1.02±0.12</td>
<td>1.09±0.15</td>
<td>1.02±0.13</td>
<td>1.04±0.13</td>
</tr>
<tr>
<td>Denervated</td>
<td>1.34±0.44</td>
<td>1.20±0.44</td>
<td>1.24±0.43</td>
<td>1.24±0.38</td>
</tr>
<tr>
<td>10%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td>1.21±0.24</td>
<td>1.10±0.34</td>
<td>1.21±0.41</td>
<td>1.20±0.35</td>
</tr>
<tr>
<td>Denervated</td>
<td>1.14±0.33</td>
<td>1.12±0.26</td>
<td>1.10±0.26</td>
<td>1.07±0.28</td>
</tr>
<tr>
<td>20%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td>0.97±0.09</td>
<td>1.03±0.21</td>
<td>1.03±0.14</td>
<td>1.05±0.13</td>
</tr>
<tr>
<td>Denervated</td>
<td>1.18±0.38</td>
<td>1.20±0.34</td>
<td>1.24±0.38</td>
<td>1.24±0.38</td>
</tr>
</tbody>
</table>

Values are means ± SD. RBF, renal blood flow; RVR, renal vascular resistance. See Experimental details for description of protocol.
RESULTS

Effects of hemorrhage in intact lambs. In intact lambs, blood pressure remained constant after 0 and 10% hemorrhage but decreased transiently at 20 min to 73 ± 7 from 84 ± 6 mmHg in response to 20% hemorrhage with control levels being reached by 40 min ($F = 4.70, P = 0.004$). Heart rate remained constant after 0% hemorrhage. There was an increase in heart rate at 60 min after 10% hemorrhage and at 20 min after 20% hemorrhage, which was sustained for 60 min (Fig. 1). There was no effect of hemorrhage on renal blood flow ($F = 0.26, P = 0.78$) or on renal vascular resistance ($F = 0.5, P = 0.95$) in intact lambs (Table 2).

Plasma renin activity was not altered by 0 or 10% hemorrhage but increased after 20% hemorrhage ($F = 6.69, P < 0.001$; Fig. 2). Hemorrhage of 0 and 10% of vascular volume did not alter plasma levels of arginine vasopressin. Hemorrhage of 20% elicited an increase in plasma levels of arginine vasopressin at 20 min (Fig. 3); control levels were reached by 60 min.

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Effects of renal denervation. In denervated lambs, basal heart rates were significantly elevated compared with those measured in intact lambs (Tables 1 and 2, Fig. 1). Baseline renal hemodynamics were not significantly altered by renal denervation (Table 2), although the variance appeared to be increased in denervated compared with intact lambs.

Baseline levels of plasma renin activity were increased in denervated compared with intact lambs ($F = 10.92, P = 0.002$; Table 1, Fig. 2), whereas plasma levels of arginine vasopressin were not altered by renal denervation ($F = 1.14, P = 0.29$; Table 1).

Effects of hemorrhage in denervated lambs. In denervated lambs, blood pressure remained constant after 0 and 10% hemorrhage. Blood pressure decreased from 87 ± 10 to 79 ± 9 mmHg within 20 min of the start of 20% hemorrhage in denervated lambs and remained significantly decreased compared with control levels at 60 min. Therefore, the recovery of blood pressure was delayed in denervated lambs ($F = 6.54, P = 0.015$) compared with the rapid return of blood pressure to control levels seen in intact lambs. There was no
further increase in heart rate after 10–20% hemorrhage in denervated lambs from the already elevated levels (Fig. 1). This response was different from that seen in intact lambs ($F = 19.26, P = 0.001$). Renal blood flow and renal vascular resistance remained unaltered by 0–20% hemorrhage in denervated lambs, as in intact lambs (Table 2).

Plasma renin activity remained constant after 0–10% hemorrhage in denervated lambs and increased by 20 min after 20% hemorrhage in denervated lambs. This increase in plasma renin activity in response to 20% hemorrhage was not different from that measured in intact lambs ($F = 0.17, P = 0.84$; Fig. 2). For plasma levels of arginine vasopressin there was, however, an interaction ($F = 3.35, P = 0.04$) between the degree of hemorrhage (0, 10, and 20%) and the presence or absence of renal sympathetic nerves, as well as an interaction ($F = 3.29, P = 0.016$) between the degree of hemorrhage (0, 10, and 20%), presence or absence of renal sympathetic nerves, and time. These results are illustrated in Fig. 3, which shows the lack of an increase in plasma levels of arginine vasopressin in denervated compared with intact lambs.

**DISCUSSION**

The present experiments were designed to investigate the role of renal sympathetic nerves in influencing cardiovascular and endocrine responses to hemorrhage in conscious, chronically instrumented newborn lambs. Our present observations showed that renal nerves modulate baseline cardiovascular and endocrine function early in life, which confirms our previous findings. Additional, novel findings of the present study are that renal nerves also appear to modulate the cardiovascular and endocrine responses to hemorrhage. Perhaps of primary importance is the abolition of hemorrhage-induced release of arginine vasopressin in the absence of renal nerves and an associated decrease in the rate of recovery of blood pressure after hemorrhage.

In conscious adult sheep (5, 6), dogs (45), and monkeys (2), hemorrhage results in an increase in plasma and cerebrospinal fluid levels of arginine vasopressin during the hypotensive phase of hemorrhage. This suggests that arginine vasopressin contributes predominantly to promoting the recovery from hemorrhagic hypotension, playing a less important role in regulating blood pressure during mild to moderate hemorrhage. This also occurs in newborn lambs, implicating a role for arginine vasopressin in assisting in the restoration of blood pressure after blood loss (4, 29, 34). Rocha E Silva and Rosenberg (28) first demonstrated in anesthetized dogs that release of arginine vasopressin in response to hemorrhage was altered when arterial baroreceptors were removed. Further studies by Cowley et al. (11) confirmed an important interaction between arginine vasopressin and the arterial baroreflex in conscious dogs. It is now well established that arginine vasopressin acts on a number of centers involved in cardiovascular regulation including the nucleus tractus solitarii, area postrema, and locus ceruleus. Arginine vasopressin exerts some of its actions by enhancing arterial baroreflex gain (3, 11, 19, 44), thereby facilitating arterial pressure. In conscious rats, rabbits, and sheep, pretreatment with an antagonist to specific vasopressin receptors alters the rate of blood pressure recovery after hypotensive hemorrhage as well as the overall tolerance to hemorrhage (14, 21, 42).

Interestingly, in the present study in conscious, chronically instrumented lambs there was also no compensatory increase in plasma levels of arginine vasopressin after hypotensive hemorrhage in the absence of renal sympathetic nerves, and, as well, the rate of blood pressure recovery after hemorrhage was decreased. This is in agreement with the above-mentioned observations in adult animals of a variety of species that the recovery of blood pressure after blood loss is dependent, at least in part, on the increased circulating levels of arginine vasopressin. Our observations therefore provide evidence that arginine vasopressin is necessary for promoting recovery from hemorrhagic hypotension early in life.

Although the mechanism of this response was not investigated in the present experiments, one can speculate that afferent renal sympathetic nerves play an important role in modulating the release of arginine vasopressin in response to hemorrhage in conscious lambs. In a variety of anatomic studies, projections...
from the kidney to the brain stem have been demonstrated. For example, Wyss and Donovan (47) provided evidence in rats that ~8% of renal afferents project monosynaptically to the lower dorsal medulla. This confirmed the observations of Simon and Schramm (31) that some myelinated fibers observed in the renal nerves of the rat transmit information via the ipsilateral fasciculus gracilis to cells in the ipsilateral nucleus gracilis and nucleus tractus solitarii. Experiments by Webb and Brody (46) provided evidence that electrical stimulation of renal afferents decreased blood pressure, thereby illustrating a physiological role for renal afferents in modulating cardiovascular homeostasis. This has been further explored and reviewed extensively by Stella and Zanchetti (41) and Ammons (1).

Caverson and Ciriello (8) demonstrated in anesthetized, paralyzed, and artificially ventilated cats that sensory information originating in renal receptors excites magnocellular neurons in the paraventricular nucleus of the hypothalamus. This observation led to the speculation that this projection may contribute to the release of arginine vasopressin during conditions in which afferent renal sympathetic nerve activity is increased. Subsequent studies in anesthetized and conscious rats and anesthetized rabbits confirmed that afferent renal sympathetic nerve stimulation is associated with a rapid increase in plasma levels of arginine vasopressin. It therefore seems likely that hemorrhage is associated with an increase in afferent renal sympathetic nerve activity, thereby leading to an increase in the release of arginine vasopressin; this would promote the recovery of blood pressure and the renal retention of fluids and electrolytes. In the absence of renal nerves, this important link is removed, thereby preventing the release of arginine vasopressin. An increase in afferent renal sympathetic nerve activity could result from a decreased renal perfusion pressure secondary to the decreased blood pressure (41), or from the direct excitatory effects of circulating humoral agents, after hypotensive hemorrhage. At this time, however, the exact mechanism underlying this response is not known.

Activation of the renin-angiotensin system occurs during the nonhypotensive phase of hemorrhage in conscious adult sheep (5, 6, 40, 43), dogs (20, 24, 45), rabbits (16, 25), and rats (14, 27). There is, however, no activation of the renin-angiotensin system after nonhypotensive hemorrhage in the first week of life in sheep (Fig. 2), implicating an age-dependent response of this system to blood loss. As blood loss continues, there is further activation of the renin-angiotensin system in adult animals (14, 25, 26). In our experiments, further blood loss associated with 20% of vascular volume resulted in activation of the renin-angiotensin system.

Experiments conducted by Nelson and Osborn (23) in conscious adult dogs were designed to determine the mechanism(s) of this renin response to hemorrhage. Their experiments showed that the increase in renin secretion that occurred after hemorrhage was abolished in the presence of bilateral renal denervation or in the presence of drugs that blocked the action of renal nerves. It was concluded (23) that the increase in renin secretion after hemorrhage was elicited by activation of renal sympathetic nerves. This is contrary to what was observed in the present study in newborn lambs, in which the increase in renin secretion after 20% hemorrhage was not altered by renal denervation. Similarly, in a previous study in conscious lambs (39), we observed that the increase in plasma renin activity following administration of furosemide also remained unaltered by renal denervation. Although it is possible that species differences are involved, this discrepancy in the responses of newborn lambs and adult animals could reflect age-dependent differences in the factors regulating renin secretion after blood loss. Further experiments are necessary to confirm this postulate.

In previous studies, we investigated the role of renal sympathetic nerves in regulating a number of physiological responses to perturbations in blood volume and blood pressure early in life (35, 38, 39). An overall finding of these investigations in conscious lambs is that renal sympathetic nerves appear to regulate the arterial baroreflex regulation of heart rate. An increase in basal heart rates after renal denervation in conscious lambs in the present study confirms our previous studies (35, 38), in which we suggested that renal afferents play an important role in modulating centers that regulate the arterial baroreflex. In the present experiments, we also observed an increase in basal blood pressures and plasma renin activity after renal denervation. These data provide evidence that renal sympathetic nerves play an important role in regulating baseline renin secretion as well as cardiovascular homeostasis early in life. We also observed an apparent increase in the variability of baseline renal hemodynamics after bilateral renal denervation. This observation may suggest a role for renal nerves in buffering renal hemodynamics under normal physiological conditions, although the physiological significance of this remains to be determined.

In conclusion, the present observations provide new information that renal sympathetic nerves appear to play an important role in regulating baseline cardiovascular and endocrine function as well as the cardiovascular and endocrine responses to hemorrhage early in life. Our findings in conscious lambs provide new evidence that arginine vasopressin plays an important role in promoting the recovery of blood pressure after blood loss early in life and that renal sympathetic nerves play a predominant role in initiating this response.

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