

## Plasma Renin, Catecholamine, and Vasopressin during Nitroprusside-induced Hypotension in Ewes

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The effect of acute nitroprusside-induced hypotension on plasma renin activity, catecholamine, and vasopressin concentrations was examined in eight chronically catheterized, conscious ewes. Nitroprusside was infused intravenously for one hour at rates adjusted to achieve a 20% decrease in mean blood pressure (dose range: 14–50 mg, or about  $5.8\text{--}18.5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ). During hypotension, renin activity increased from  $1.39 \pm 0.49$  to  $3.92 \pm 1.38 \text{ ng} \cdot \text{ml}^{-1} \cdot \text{h}^{-1}$ , catecholamine concentrations remained unchanged, and vasopressin increased from  $1.7 \pm 0.4$  to  $110 \pm 52.7 \text{ pg/ml}$ . A significant positive correlation was obtained between total nitroprusside dose and peak vasopressin level ( $r = 0.749$ ,  $P = 0.015$ ). No significant change in arterial-blood pH,  $P_{\text{O}_2}$ ,  $P_{\text{CO}_2}$ , plasma osmolality, or sodium concentration were observed throughout the experiment, thus eliminating the possibility of osmolar or hypoxic stimuli for the increased renin activity and vasopressin release. The magnitude of vasopressin release found in our studies implies that it plays a more important role than renin in defense against acute hypotension. In addition, the authors experiments suggest that variation in vasopressin release may be responsible for the variation of the dose of nitroprusside required to maintain hypotension. (Key words: Anesthetic techniques: hypotension, nitroprusside. Hormones: vasopressin. Polypeptides: renin-angiotensin. Sympathetic nervous system: catecholamines.)

NITROPRUSSIDE recently has gained popularity as a hypotensive agent for treatment of hypertensive crisis, intraoperative blood pressure manipulation, afterload reduction, and severe congestive heart failure.<sup>1</sup> The drug appears to produce relaxation of the smooth muscles by interacting directly with sulfhydryl groups on the vascular smooth muscle membrane.<sup>2</sup>

Clinical studies have demonstrated an interpatient variation in dose of nitroprusside required to maintain a lowered blood pressure.<sup>1,3,4</sup> Since release of vasoactive agents, including vasopressin, is known to occur in response to the lowering of blood pressure or decrease in effective blood volume,<sup>5,6</sup> it can be speculated that individual variability in response to nitroprusside may involve variability in the release of vasoactive mediators. The present experiments in ewes were undertaken to investigate the effect of hypotension induced by nitro-

prusside on plasma levels of renin, catecholamines, and vasopressin and the potential role of these mediators on the variability in drug dose required to induce a predetermined reduction in blood pressure.

### Materials and Methods

Eight, healthy, mixed-bred adult ewes, 2–4 years old (40–45 kg), with chronically indwelling femoral catheters (placed via femoral cut down) were studied at least 1 week after surgery. After a 30-min control period of blood pressure and heart rate measurements, freshly prepared nitroprusside (0.2 mg/ml in 0.9% saline) was infused intravenously. The rate of infusion was adjusted so that the mean blood pressure decreased by 10 to 20 mmHg within the first 10 min of the infusion. The infusion was continued for a total of 60 min with the rate being adjusted to maintain a constant mean blood pressure during the study period. The total dose of nitroprusside necessary to achieve these pressure reductions varied from 14 to 50 mg per animal (about  $5.8\text{--}18.5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ). (Thus, the total dose was less than  $1.5 \text{ mg} \cdot \text{kg}^{-1}$  and therefore below the level at which cyanide toxicity is seen in other species.<sup>3</sup>)

Arterial blood samples for the various determinations were taken before, during, and following the infusion (total blood withdrawal of 30 ml per study). This amount of blood drawn over a two-hour period has been shown not to affect vasopressin levels.\*\* Blood pH and gas tensions were measured immediately using microelectrodes and a Radiometer blood-gas monitor.<sup>7</sup> Plasma was separated after centrifugation at  $2,000 \times g$  at  $4^\circ \text{C}$ , and stored at  $-30^\circ \text{C}$  for later determination of renin, catecholamines, vasopressin, electrolytes, and osmolality.

Arterial blood pressure was measured using a Statham transducer; heart rate was determined by a cardiometer triggered by pulse pressure. These data were recorded on a multichannel Beckman® polygraph.

Samples for renin and catecholamine were collected in chilled tubes containing EGTA and glutathione. Renin activity was measured by generation of angiotensin I using New England Nuclear® Rianen™ angiotensin I [ $^{125}\text{I}$ ] Radioimmunoassay Kit; the sensitivity is ap-

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proximately 2 pg per sample with a precision of  $0.55 \pm 0.11$  for values less than  $2 \text{ ng} \cdot \text{ml}^{-1} \cdot \text{h}^{-1}$ , and  $7.40 \pm 0.81$  for values  $7\text{--}10 \text{ ng} \cdot \text{ml}^{-1} \cdot \text{h}^{-1}$ . Epinephrine and norepinephrine were measured by a radioenzymatic method using Upjohn's Cat-A-Kit<sup>TM</sup>. Sensitivity of Upjohn's method ranges from 2–5 pg per 50  $\mu\text{l}$  for both epinephrine and norepinephrine. The average coefficient of variation within standard runs was 10% for epinephrine and 7.5% for norepinephrine.

Samples for determination of vasopressin were collected in chilled tubes containing EDTA. Concentrations were determined by radioimmunoassay as described previously.<sup>8</sup> The assay can detect 0.2 pg/ml of vasopressin. Interassay variation, as determined on five successive assays of pooled plasma, was 17.3%, whereas intraassay variation, as determined by the 50% intercept point in the standard curves, was 8.4%.

In six of the eight animals, blood was obtained for serum sodium and osmolality and was measured using an Instrumentation Lab Flame Photometer and an Advanced Instrument Hi-Precision Research Osmometer.

One of the animals had a blood pressure during the control period that was greater than two standard deviations from the mean of the seven other animals; the etiology was unknown. However, the heart rate as well as the vasoactive mediators which were measured, were within the range of the data obtained from the seven other animals. Therefore, the data from all animals were analyzed together. To eliminate large standard deviations when blood pressure data were evaluated, results were calculated as change in mean blood pressure from the control period.

Statistical significance was analyzed by the Student's two-tailed *t* test for paired samples using the control pre-infusion period for comparison with experimental points. The control period for blood pressure and heart rate, designated as time zero, represents an average of at least four points during the 30 min prior to the nitroprusside infusion. Linear regression analysis was used to correlate total nitroprusside dose with peak vasopressin concentration. Analysis of variance for fixed random design was used to determine significance for differences in vasopressin, renin, epinephrine, norepinephrine, and total catecholamine concentrations. Results are reported as mean  $\pm$  SE unless otherwise stated.

### Results

Figure 1 shows the changes in mean blood pressure from the control period ( $\Delta\text{BP}$ ). Blood pressure fell significantly below control values ( $90 \pm 7 \text{ mmHg}$ ) ( $P < 0.001$ ) 2 min after the infusion started until 2 min after the infusion ended, with the nadir being reached from 10 through 30 min.

Although the mean value for heart rate increased from 108 to 132 bpm during nitroprusside-induced hypotension (Fig. 1), this change was not statistically significant.

The changes in vasoactive mediators are depicted graphically in figure 1. Renin activity increased from control values of  $1.39 \pm 0.49$  to  $3.92 \pm 1.38 \text{ ng} \cdot \text{ml}^{-1} \cdot \text{h}^{-1}$  at 60 min ( $P < 0.05$ ). On the other hand, the increase in total catecholamine concentrations (epinephrine plus norepinephrine) from control values of  $172.1 \pm 58.4$  to  $240.8 \pm 111.1 \text{ pg/ml}$  was not statistically significant. Vasopressin concentrations increased more than fifty-fold during the nitroprusside infusion, from a control value of  $1.7 \pm 0.4$  to  $110.0 \pm 52.7 \text{ pg/ml}$  after 60-min infusion ( $P < 0.01$ ). There was a positive correlation between total nitroprusside dose and peak vasopressin level ( $r = 0.75$ ,  $P = 0.015$ ).

There were no statistically significant differences in the mean arterial pH and blood-gas tensions at intervals during and after the infusion when compared with control levels (table 1). Mean plasma sodium ( $144 \pm 1.29 \text{ mEq/l}$ ) and osmolality ( $298 \pm 3.46 \text{ mOsm/kg}$ ) were within the normal range for sheep and remained unchanged throughout the experiment.

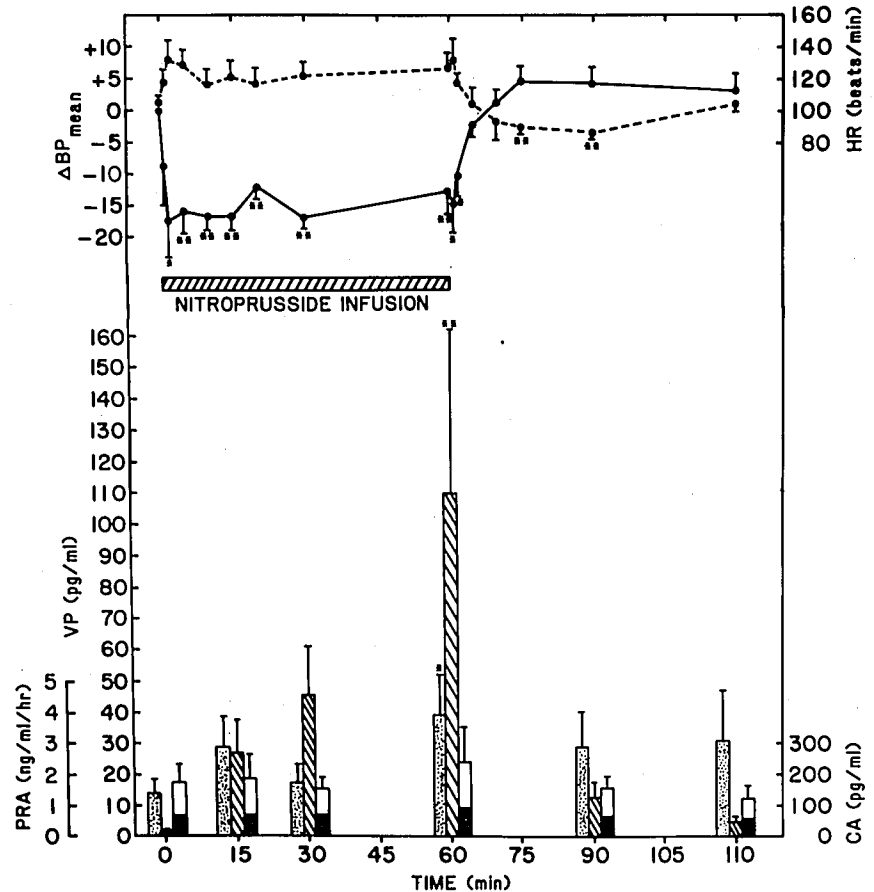
### Discussion

The experiments demonstrate that when nitroprusside is given to the conscious, healthy, normotensive ewe in doses that lower mean blood pressure by 10–20 mmHg, the animal responds with a doubling of her renin activity and a greater than fifty-fold increase in vasopressin concentration, while plasma epinephrine, norepinephrine, arterial pH, blood gases, sodium, and osmolality remain unchanged.

Consistent with findings in other species, including humans<sup>1,4,9,10</sup> this study shows a significant fall of blood pressure immediately after the introduction of nitroprusside. As noted by others, this was followed by a mild rebound hypertension upon cessation of the drug, then a return to control values.<sup>11,12</sup> This rebound could be eliminated by propranolol, nephrectomy, or converting enzyme blockade<sup>12</sup> and, therefore, presumably is influenced by the sympathetic nervous system and the renin-angiotensin system. In addition, vasopressin may play an important role.

The effect of nitroprusside on heart rate appears to be controversial.<sup>4</sup> Some investigators using different species,<sup>9,10,12</sup> have observed tachycardia during hypotension. We observed no statistically significant changes. Fahmy<sup>13</sup> has postulated the effect of nitroprusside on heart rate reflects the predominant autonomic tone at the time of induced hypotension. Support for the au-

FIG. 1. Time 0 represents the control period. (Upper portion): Change in mean blood pressure from control period ( $BP_{mean}$ , mmHg) (solid line) and heart rate (HR, beats/min) (broken line) before, during, and following infusion of nitroprusside (Mean  $\pm$  SE). (Lower portion): Bar graph representing plasma renin activity (PRA,  $ng \cdot ml^{-1} \cdot h^{-1}$ ) (stippled bar on the left), vasopressin (VP, pg/ml) (hatched bar in the middle), and catecholamine (CA, pg/ml) (clear plus solid bar on the right) before, during, and following infusion of nitroprusside. The catecholamine level shows the contribution of epinephrine (clear upper portion) and norepinephrine (solid lower portion) to the total level (Mean  $\pm$  SE). \* $P < 0.05$ ; \*\* $P < 0.01$ .



tonomic nervous system's role in promotion of tachycardia comes from the observation that propranolol will block an increase of heart rate.

With the use of various drugs (hydralazine,<sup>14,15</sup> minoxidil,<sup>15</sup> nitroprusside<sup>11,12,16</sup>) to induce hypotension, observations have confirmed a role for the renin-angiotensin system for blood pressure maintenance. Our results agree with the findings of other investigators by demonstrating a rise in plasma renin activity during induced hypotension. Increased renin activity would generate additional angiotensin, a known potent vasoconstrictor, and, therefore, an appropriate physiologic response to hypotension. The pathophysiology of renin

release during rapidly induced hypotension may be explained by neural mediation<sup>12</sup> and/or a reflex feedback mechanism whereby angiotensin stimulates catecholamine release<sup>17</sup> which in turn stimulates additional renin release.<sup>17,18</sup> In addition, higher than expected plasma levels may be seen because nitroprusside diminishes mesenteric blood flow<sup>19</sup> and therefore presumably decreases hepatic renin metabolism.<sup>20</sup> High renin levels observed during hypotension may be explained by the above pathophysiology. Similarly, after the abrupt cessation of nitroprusside-induced hypotension, the clinically observed rebound hypertension with its accompanying reflex bradycardia may reflect renin activity

TABLE 1. Acid-base Indices (Mean  $\pm$  SE) Before, During, and After Infusion of Nitroprusside

	Time (min)			
	0	10	60	90
pH	7.46 $\pm$ 0.013	7.46 $\pm$ 0.016	7.46 $\pm$ 0.014	7.44 $\pm$ 0.015
P <sub>O<sub>2</sub></sub> (mmHg)	104 $\pm$ 5	98 $\pm$ 5	103 $\pm$ 4	102 $\pm$ 4
P <sub>C<sub>O<sub>2</sub></sub></sub> (mmHg)	34 $\pm$ 1	33 $\pm$ 2	32 $\pm$ 2	33 $\pm$ 2
BE (mEq/l)	-1.1 $\pm$ 0.7	-1.8 $\pm$ 0.8	-1.4 $\pm$ 0.8	-0.6 $\pm$ 0.8

resulting from its prolonged half life (relative to nitroprusside) by a mechanism like that described above.

Rawlinson *et al.*<sup>21</sup> reported a significant increase in plasma concentration of epinephrine and norepinephrine during nitroprusside infusion which correlated with the per cent decrease in mean blood pressure in five normal patients while under anesthesia. (In nine other patients with subarachnoid hemorrhage, who also were made hypotensive, he found no change in plasma catecholamine concentrations.) He postulated a reflex increase in sympatho-adrenal medullary activity. The difference in our observation from those of Rawlinson *et al.*<sup>21</sup> may be explained by his small sample size, use of general anesthesia, a greater change in blood pressure, or species differences.

Vasopressin release is known to be stimulated by hypotension and hypovolemia.<sup>22</sup> Although we made animals hypotensive without directly altering their blood volume, nitroprusside had been reported to produce intravascular volume depletion by sudden pooling of blood because of dilation of capacitance vessels.<sup>10</sup> Infusion of nitroprusside provoked a release of vasopressin to levels 50 times greater than control at the end of one hour. Levels this high have been shown to act as a vasopressor and, therefore, vasopressin secretion would act as a defense against hypotension.<sup>23</sup> Robertson<sup>6</sup> observed a curvilinear relationship between decline of mean arterial blood pressure and increase in vasopressin secretion; the greater the fall in blood pressure, the more vasopressin was secreted. Controlled hemorrhage also will cause vasopressin release,<sup>24-28</sup> although debate exists between the role of hypovolemia,<sup>25,26</sup> and hypotension.<sup>24,26</sup> Laycock *et al.*<sup>28</sup> have shown that after hemorrhage, vasopressin secretion is needed to return the blood pressure back towards control.

Arnauld *et al.*<sup>24</sup> postulated that the rapid vasopressin release observed during hemorrhage suggests that nervous rather than humoral factors are the key stimulators for vasopressin release. Furthermore, baroreceptors are more important than atrial receptors. In addition, it seems impulses for the sinoaortic and vagus nerves are both needed for the regulation of vasopressin release.<sup>29</sup>

No change was observed in acid-base status during the entire protocol, implying that significant cyanide toxicity was not present; furthermore, hypoxia and acidosis were not major stimuli to catecholamine or vasopressin release. In addition, sodium and osmolality also were found to be unchanged. This excludes the possibility that vasopressin release was stimulated by hemoconcentration.

In summary, nitroprusside is an effective agent in producing systemic hypotension in the ewe. Although in our experiments the plasma catecholamine concen-

trations remained unchanged, this does not minimize the role of the autonomic nervous system in blood pressure regulation. The ewe seemingly attempts to preserve her blood pressure by the release of renin and vasopressin. Because the magnitude of the vasopressin response is so much greater than the increase in renin activity, we speculate that vasopressin is more important in the defense against acute hypotension in the sheep. The reason for the variability in the release of vasopressin, as well as the interactions with still other vasoactive mediators, remains to be examined. Vasopressin concentration in our animals correlated with the total nitroprusside dose required to maintain a lowered blood pressure. This observation supports our speculation that variation in the nitroprusside dose required to maintain hypotension in the ewe may in part, be caused by the individual variation in vasopressin and renin release.

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