PRESSOR and vascular hyperresponsiveness to vasoconstrictor substances has been reported in animal models of hypertension that are presumed to have expanded body fluid volumes, such as rats treated with deoxycorticosterone acetate (DOCA) and given a high sodium intake (DOCA-salt hypertension) and one-kidney rats with renal artery stenosis (1K1C). It has been proposed that natriuretic hormone may be involved in mediating the hyperresponsiveness in these volume-expanded states. However, pressor and vascular hyperresponsiveness has also been seen in two-kidney rabbits with unilateral renal artery stenosis (2K1C rabbits), a hypertensive animal model with normal body fluid volumes. Furthermore, in this 2K1C model and in other hypertensive animal models involving renal perturbations, angiotensin II (ANG II) has been shown to play an essential role in mediating the pressor and vascular hyperresponsiveness, even though plasma renin activity (PRA) and plasma ANG II concentrations were not elevated.

Our present study examined whether expansion of body fluid volumes would result in pressor and vascular hyperresponsiveness. This was determined by testing the pressor responses to norepinephrine (NE) in rabbits that were volume-expanded by the intravenous (i.v.) infusion of isotonic saline for 24 hours. This study also examined whether ANG II might be involved in mediating enhanced pressor responses in volume-expanded animals. This was achieved by determining the effect of an ANG II-competitive antagonist on the pressor responses to NE in these saline-infused rabbits. In an additional series of experiments that investigated the relative contributions of changes in cardiac output (CO) and total peripheral resistance (TPR) in the pressor hyperresponsiveness with volume expansion, the effect of NE infusion on cardiac output (CO) was determined both before and after saline infusion.

SUMMARY Conscious rabbits infused intravenously (i.v.) with isotonic saline at 1.5 to 1.8 ml/min for 24 hours had greater pressor responses to norepinephrine (NE) than did normal control rabbits. Infusion of the angiotensin II (ANG II) antagonist [Sar\(^1\), Ile\(^8\)] ANG II did not decrease the exaggerated pressor responses to NE in saline-infused rabbits. Measurements of cardiac output (CO) as well as the pressor responses to NE before and after saline infusion revealed that, although saline infusion increased the CO and decreased total peripheral resistance (TPR), CO did not change during NE infusion either before or after saline infusion, but NE produced significantly greater increases in mean arterial pressure (MAP) and TPR after saline infusion than before the saline infusion. The cross-circulation of blood at 10 ml/min for 5 minutes between saline-infused donor rabbits and normal recipient rabbits resulted in pressor hyperresponsiveness to NE in the normal recipients. Similar cross-circulation experiments between pairs of normal rabbits did not alter the pressor responses to NE. These studies provided direct evidence that expansion of body fluid volumes by saline infusion results in pressor and vascular hyperresponsiveness. There was no evidence to indicate that ANG II was involved in the mechanisms producing this pressor hyperresponsiveness. Some circulating hormonal factor, however, was involved in mediating the pressor hyperresponsiveness following saline infusion. The results of this study are compatible with the concept that natriuretic hormone may play a role in promoting pressor hyperresponsiveness in saline-expanded animals.

(HTN 6: 503-510, 1984)

**KEY WORDS** angiotensin II antagonist • [Sar\(^1\), Ile\(^8\)] angiotensin II • cardiac output • blood cross-circulation • hormonal factors

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Pressor Hyperresponsiveness in Saline-Infused Rabbits

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Pressor hyperresponsiveness has also been seen in two-kidney rabbits with unilateral renal artery stenosis (2K1C rabbits), a hypertensive animal model with normal body fluid volumes. Furthermore, in this 2K1C model and in other hypertensive animal models involving renal perturbations, angiotensin II (ANG II) has been shown to play an essential role in mediating the pressor and vascular hyperresponsiveness, even though plasma renin activity (PRA) and plasma ANG II concentrations were not elevated.

Our present study examined whether expansion of body fluid volumes would result in pressor and vascular hyperresponsiveness. This was determined by testing the pressor responses to norepinephrine (NE) in rabbits that were volume-expanded by the intravenous (i.v.) infusion of isotonic saline for 24 hours. This study also examined whether ANG II might be involved in mediating enhanced pressor responses in volume-expanded animals. This was achieved by determining the effect of an ANG II-competitive antagonist on the pressor responses to NE in these saline-infused rabbits. In an additional series of experiments that investigated the relative contributions of changes in CO and TPR in the pressor hyperresponsiveness with volume expansion, the effect of NE infusion on cardiac output (CO) was determined both before and after saline infusion.
nally, this study examined the hypothesis that a circulating humoral factor is involved in mediating pressor hyperresponsiveness in volume-expanded animals. This was accomplished by determining the pressor responses to NE before and after the cross-circulation of blood between saline-infused rabbits and normal rabbits.

**Methods**

We used 48 male New Zealand white rabbits that weighed from 2.6 to 3.6 kg. All rabbits were housed one per cage in a room in which temperature was kept at 27°C and lighting was controlled automatically on a 12-hours-on (7:00 a.m. to 7:00 p.m.) and 12-hours-off basis. The rabbits were fed an excess of a commercial rabbit diet (Purina Lab Rabbit Chow HF5326) that contained 0.167 mEq of Na+ and 0.467 mEq of K+ per gram of feed. Water was available ad libitum. All rabbits were housed for at least 1 week prior to any experiments.

On the day before the experiment, rabbits that were to be saline-infused were anesthetized with 2% to 5% of halothane in nitrous oxide and oxygen, as described by Sartick et al.2 Sterile surgical procedures were used to expose the bladder through a ventral midline incision and insert a polyvinyl catheter (made from a French 8 infant feeding tube by placing a collar of silicone rubber approximately 2½ cm from the end) through a small incision in the bladder wall. The catheter was placed so that the collar was located inside the bladder to prevent it from slipping. The bladder incision was closed by a purse-string suture, and the abdominal incision was closed. The catheter was cut off so that only 3 cm protruded from the ventral abdominal wall. This portion then was split lengthwise as far as the abdominal wall, and the free ends were splayed and sutured to the abdominal wall. These procedures allowed the urine to drain continuously from the urinary bladder to the extremum. Polyvinyl catheters (French 5 infant feeding tubes) were also placed surgically into the femoral artery and vein. These rabbits were then placed in boxes and used in acute experiments 6 hours later. During the acute experiments, the conscious rabbits appeared undisturbed and did not display any evidence of discomfort.

At the beginning of some experiments, an arterial blood sample of 3 ml was obtained from each rabbit for the measurement of plasma renin activity (PRA). This sample was placed in a chilled tube containing ethylenediaminetetraacetic acid (EDTA) and spun in a refrigerated centrifuge at 4°C. The plasma was stored frozen at −14°C until processed and assayed. PRA was determined by radioimmunoassay of generated angiotensin I (ANG I) by a modification of the method of Cohen et al.10 This assay, as performed in our laboratory, has been described previously.11 The MAP was measured during each experiment by means of a pressure transducer (Statham, Model P23Db) attached to the femoral arterial catheter. Arterial pressures were recorded on an oscillographic recorder (Hewlett-Packard Model 7754A). During the recording of responses of MAP to NE infusions, we increased the gain of the recorder amplifier so that a change in arterial pressure of 1 mm Hg would produce a pen deflection of 1 mm. We also adjusted the baseline of the pressure recording by means of a zero-suppression control. These procedures allowed accurate measurements of small changes in MAP. The HR was obtained by recording pulsatile arterial pressure at a fast paper speed.

The CO was determined in one series of experiments by dye dilution, as has been described previously.11 Each rabbit was heparinized (1000 units). Blood was pumped from the arterial catheter through a densitometer cuvette (Model DC-410, Waters Instruments, Rochester, Minnesota) at a rate of 10.0 ml/min by a roller pump. The blood was then returned to the rabbit through the femoral venous catheter. A volume of 0.15 ml of indocyanine green dye (Cardio-Green; Hynson, Westcott, and Dunning, Inc., Baltimore, Maryland), with a dye concentration of 2.5 mg/ml, was placed in the jugular catheter and flushed into the circulation with 0.8 ml of isotonic saline. The densitometer cuvette was interfaced with a densitometer (Model TD-1, Waters Instruments, Rochester, Minnesota), and the dye concentration curves were recorded on the oscillographic recorder. All CO determinations were performed in triplicate, and the average of the three determinations was accepted as the CO value.

In the saline infusion experiments in which sodium balances were determined, the total urine output was collected during the saline infusion in each rabbit, and the volume of urine was measured. Urine sodium con-
centrations were determined by flame photometry. Sodium excretions were calculated by multiplying the urine volume times the urine sodium concentration.

Three types of experiments were performed. The first experiment examined the pressor responses to NE in saline-infused rabbits, without and with the concurrent i.v. infusion of the ANG II antagonist [Sar^1, Ile^8] ANG II. The second experiment measured the effect of a single dose of NE on MAP and CO both before and after saline infusion. The third experiment tested for the presence of a blood-borne humoral factor that might mediate the pressor hyperresponsiveness in saline-infused rabbits by the cross-circulation of blood between these rabbits and normal recipient rabbits.

Experiment 1: Pressor Responses to Norepinephrine

This experiment used three groups of six rabbits each. The first group consisted of six normal rabbits; and the second group, of six rabbits that were saline-infused. The third group of six rabbits was also infused with saline but received an i.v. infusion of [Sar^1, Ile^8] ANG II in addition. The third group of rabbits had a catheter of polyethylene tubing (PE 50) placed percutaneously into the marginal ear vein 30 to 60 minutes before the start of the experiment. This catheter was used for infusion of the ANG II antagonist. At the start of each experiment, an arterial blood sample was collected for the determination of PRA, and control measurements of MAP and HR were obtained. In the third group of rabbits, a bolus injection of 300 ng of ANG II was made through the ear catheter, and the pressor response was recorded. For the remainder of the experiment, these rabbits received an i.v. infusion of a saline solution of [Sar^1, Ile^8] ANG II at a dose rate of 300 ng/min/kg body wt and at a flow rate of 0.34 ml/min. After 30 minutes of infusion of the ANG II antagonist, the pressor response to an i.v. injection of 300 ng of ANG II was again obtained in these rabbits.

In all rabbits in this experiment, solutions of NE were infused through the femoral venous catheter in doses of 25, 50, 100, 200, 400, 800, and 1200 ng/min/kg per kg of body weight. Each NE dose was infused for 5 minutes at a flow rate of 0.34 or 0.68 ml/min, and the pressor responses were recorded. During the 1-minute period prior to each infusion the MAP was taken as the control pressure, and the increase in MAP during the infusion was taken as the pressor response for that NE dose. At least 5 minutes were allowed between NE infusions, and the next dose of NE was not infused until the MAP had returned and stabilized to approximately the original control pressure. The solutions of NE for infusion were prepared by diluting a commercial stock solution of NE (Levophed; Breon Laboratories, Sterling Drug Inc., New York, New York) with 5% dextrose in water. A new ampule of NE was used for each experiment.

Experiment 2: Cardiac Responses to Norepinephrine

Six rabbits were used in this experiment. All rabbits had a catheter placed in the bladder and catheters also placed in the femoral artery and vein and jugular vein.

Six hours later, each rabbit was weighed, and an arterial blood sample was obtained for hematocrit and for plasma Na\(^+\) and K\(^+\) concentrations. The MAP, HR, and CO were measured. A solution of NE then was infused i.v. at 800 ng/min/kg body wt for 5 minutes, and the pressor response was recorded; HR and CO were again determined during the final minute of NE infusion. The jugular and femoral arterial catheters were then filled with a heparin solution, and the free ends of all vessel catheters were taped to the dorsal side of the animal. Each rabbit was placed in a box with a wire mesh bottom which was then placed in a metabolic cage, and isotonic saline was infused i.v. for 24 hours, as in Experiment 1. At the conclusion of the saline infusion, each rabbit was again weighed, placed in a standard rabbit box, and returned to the laboratory. An arterial blood sample was obtained for hematocrit and for plasma concentrations of Na\(^+\) and K\(^+\). After initial measurements of MAP, HR, and CO, NE was again infused i.v. at 800 ng/min/kg body wt for 5 minutes, and the increase in MAP was recorded. As before, HR and CO were determined during the last minute of NE infusion.

Experiment 3: Cross-Circulation of Blood

For Experiment 3, rabbits were used in pairs; for each pair one was the donor and the other was the recipient. Two groups of experiments were performed. In the first group of experiments, six saline-infused rabbits served as donors, and six normal rabbits were the recipients. In the second group of experiments, six normal rabbits were the donors, and six normal rabbits were the recipients. Before the rabbits were infused with saline, they were weighed. An arterial blood sample (1 ml) was obtained for the determination of hematocrit; MAP and HR also were measured. These rabbits then received an infusion of a solution of NE i.v. at 800 ng/min/kg body wt for 5 minutes, and the pressor response to this NE infusion was recorded. Isotonic saline then was infused i.v. for 24 hours, as in Experiment 1. At the conclusion of the saline infusion, these rabbits were again weighed, and the hematocrit was determined.

At the start of the acute experiment, an arterial blood sample was obtained from all donor and recipient rabbits for the measurement of PRA. The pressor response to an i.v. infusion of NE at 800 ng/min/kg body wt was obtained for all rabbits. Then the NE solution was cleared from the venous catheter, and each rabbit was given an i.v. injection of 1000 units of heparin. The femoral catheters of the donor and recipient rabbit of each pair were connected to the tubing of a single-roller, dual-chamber infusion pump so that in one chamber the blood would be pumped from the femoral artery of the donor rabbit into the femoral vein of the recipient, and in the other chamber the blood would be pumped from the femoral artery of the recipient rabbit to the femoral vein of the donor. The tubing of each chamber had a volume capacity of approximately 3 ml and was filled with sterile isotonic saline before being connected to the rabbit catheters.
The cross-circulation of blood then was achieved by starting the pump. The pump speed was adjusted so that the rate of blood exchange between the pair of rabbits was 10.0 ml/min. Because both pump chambers were connected to the same roller, the rate of exchange of blood between the two rabbits was the same in each pair. Blood was cross-circulated for 5 minutes. An arterial blood sample was then obtained from all saline-infused rabbits and their recipients for hematocrit measurements. In all of the experiments, at the completion of the blood cross-circulation, the arterial catheter of the recipient rabbit was connected again to the pressure transducer, and MAP was recorded. Pressor responses to NE infused again at 800 ng/min/kg body wt were determined in all recipient rabbits at 5, 15, 25, and 35 minutes after the termination of the cross-circulation.

Statistical Analysis

Initial values for MAP, HR, and PRA, and the pressor responses to the infusion of each dose of NE were compared among the three groups of rabbits in Experiment 1 by analysis of variance. When significant values were observed, the data were analyzed further by Duncan’s new multiple range test. In rabbits infused with saline in Experiments 2 and 3, the values for body weight, hematocrit, MAP, and the pressor response to NE after saline infusion were compared to the values before saline infusion by Student’s t test for paired observations. Likewise, in Experiment 2 the values for CO, stroke volume, TPR, and the changes in these values in response to NE infusion after saline infusion were compared to values before saline infusion by Student’s t test for paired observations. Values for plasma concentrations of Na\(^+\) and K\(^+\) in Experiment 2 were also compared before and after saline infusion by Student’s t test for paired observations. Initial values for MAP, HR, PRA, and the pressor responses to infusions of NE were compared between the donor rabbits and the recipient rabbits in each of the two groups in Experiment 3 by Student’s t test for group observations. The pressor responses to NE in the recipient rabbits in Experiment 3 at the various times after blood cross-circulation were analyzed by the U test to determine if the values were significantly different from the pressor responses prior to cross-circulation. Significance levels of \(p < 0.05\) and \(p < 0.01\) were selected for all statistical evaluations.

Results

Analysis of the data on fluid and sodium balances during saline loading for the 12 rabbits in which these were determined showed that an average volume of 2330 \(\pm\) 139 (SEM) ml of saline was infused during the 24-hour period. The volume of urine excreted during this time was 1820 \(\pm\) 147 ml. Sodium intake by saline infusion averaged 359 \(\pm\) 21 mEq, while urinary sodium excretion was 270 \(\pm\) 21 mEq, for a net sodium gain of 89 \(\pm\) 8 mEq. The average body weight of these rabbits was 2.88 \(\pm\) 0.05 kg before saline infusion and 3.03 \(\pm\) 0.06 kg after saline infusion, a significant increase \((p < 0.01)\). The hematocrit before saline infusion averaged 0.40 \(\pm\) 0.01, which decreased significantly \((p < 0.01)\) to 0.32 \(\pm\) 0.01 after saline infusion. Values for MAP, HR, and PRA in the recipient rabbits in each of the two groups were not significantly different from the normal group.

Figure 1 is a logarithmic plot of the various doses of NE vs the pressor responses produced by these doses for all three groups of rabbits in Experiment 1. These

<table>
<thead>
<tr>
<th>Rabbit group</th>
<th>Mean arterial pressure (mm Hg)</th>
<th>Heart rate (bpm)</th>
<th>Plasma renin activity (ng ANG I/ml/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>97 (\pm) 3</td>
<td>235 (\pm) 9</td>
<td>3.1 (\pm) 0.6</td>
</tr>
<tr>
<td>Saline-infused</td>
<td>95 (\pm) 3</td>
<td>208 (\pm) 16</td>
<td>2.2 (\pm) 0.2</td>
</tr>
<tr>
<td>Saline-infused plus [Sar(^1), Ile(^8)] ANG II</td>
<td>98 (\pm) 4</td>
<td>202 (\pm) 18</td>
<td>2.5 (\pm) 0.2</td>
</tr>
</tbody>
</table>

Values are means \(\pm\) SEM for six rabbits per group. There were no significant differences in values among the three groups.

Figure 1. Experiment 1: Pressor responses (mm Hg) to infusions of norepinephrine (ng/min/kg body wt; logarithmic scale) in three groups of rabbits. Solid circles and solid line = control rabbits; open circles and dotted line = saline-infused rabbits; solid squares and dot-dashed line = saline-infused rabbits receiving [Sar\(^1\), Ile\(^8\)] ANG II. Values for PRA, although slightly less in both saline-infused groups in this experiment, were not significantly different from the normal group.
dose-response relationships can be described adequately by the equation $y = a(\log x)^n$, where $y$ is the increase in MAP, $x$ is the infused dose of NE, and $a$ and $n$ are constants. The curves in this figure are the least-squares fit of this equation to the values obtained for each group of rabbits. The increases in MAP in response to NE were significantly greater in the saline-infused rabbits (both without and with [Sar$^1$, Ile$^8$] ANG II) than in the control rabbits. There were no significant differences in any of the pressor responses to NE between the saline-infused rabbits receiving [Sar$^1$, Ile$^8$] ANG II and the saline-infused rabbits not receiving it.

For Experiment 2, values for the cardiovascular measurements and the plasma concentrations of sodium and potassium both before and after saline infusion are given in Table 2. Changes in the cardiovascular values with the infusion of NE before and after saline infusion in these rabbits are summarized in Table 3. Values for CO are expressed in ml/min/kg body wt. Stroke volume is given in ml/kg of body weight. The values for TPR are expressed in the arbitrary units that resulted from dividing the MAP in mm Hg by the CO in ml/min/kg body wt. Saline infusion did not alter MAP or HR significantly but did produce a sizable increase in stroke volume. This translated into a significant decrease in TPR and an increase in stroke volume with saline infusion. Plasma sodium concentration was not significantly altered, but plasma potassium concentration was significantly decreased by saline infusion. After the rabbits were infused with saline, NE administration resulted in significantly larger increases in MAP and in TPR than occurred in response to NE before saline infusion. The magnitudes of the changes in HR and stroke volume in response to NE were the same after as before saline infusion.

The initial values for MAP, HR, and PRA for the rabbits in Experiment 3 are given in Table 4; there were no significant differences between the donor rabbits and their corresponding recipients for any of these factors. The pressor response to the i.v. infusion of NE at 800 ng/min/kg in the saline-infused rabbits averaged 19 ± 1 mm Hg, which was significantly ($p < 0.01$) greater than the pressor response of 13 ± 1 mm Hg observed prior to saline infusion. After saline infusion, the rabbits in this experiment had an average hematocrit of 0.32 ± 0.01; after the cross-circulation of blood with normal recipient rabbits the hematocrit in the saline-infused donors was unchanged at 0.34 ± 0.01. The hematocrit in the normal recipient rabbits averaged 0.37 ± 0.01, and after cross-circulation with saline-loaded rabbits the hematocrit in these normal

**Table 2. Values Before and After Saline Infusion in Rabbits in Experiment 2**

<table>
<thead>
<tr>
<th>Value</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>98±2</td>
<td>93±2</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>244±9</td>
<td>263±10</td>
</tr>
<tr>
<td>Cardiac output (ml/min/kg)</td>
<td>182±10</td>
<td>258*</td>
</tr>
<tr>
<td>Total peripheral resistance (mm Hg-min/kg/ml)</td>
<td>0.55±0.03</td>
<td>0.36*±0.01</td>
</tr>
<tr>
<td>Stroke volume (ml/kg)</td>
<td>0.75±0.05</td>
<td>1.01*±0.08</td>
</tr>
<tr>
<td>Plasma Na$^+$ (mEq/liter)</td>
<td>141.8±0.8</td>
<td>146.6±1.6</td>
</tr>
<tr>
<td>Plasma K$^+$ (mEq/liter)</td>
<td>4.0±0.1</td>
<td>3.2±0.1</td>
</tr>
</tbody>
</table>

Values are means ± SEM for six rabbits.

* $p<0.01$, that the after-saline infusion value differs from the before-saline infusion value.

† $p<0.05$, that the after-saline infusion value differs from the before-saline infusion value.

**Table 3. Changes During Norepinephrine Infusion Before and After Saline Infusion in Rabbits in Experiment 2**

<table>
<thead>
<tr>
<th>Value</th>
<th>Saline infusion Before</th>
<th>Saline infusion After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>+14*</td>
<td>+24*†</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>−68*</td>
<td>−81*†</td>
</tr>
<tr>
<td>Cardiac output (ml/min/kg)</td>
<td>−4</td>
<td>−22</td>
</tr>
<tr>
<td>Total peripheral resistance (mm Hg-min/kg/ml)</td>
<td>+0.09*</td>
<td>+0.14*†</td>
</tr>
<tr>
<td>Stroke volume (ml/kg)</td>
<td>+0.28*</td>
<td>0.59</td>
</tr>
</tbody>
</table>

Values are means ± SEM for six rabbits.

* $p<0.01$, that the change during norepinephrine infusion was significant.

† $p<0.01$, that the magnitude of the change after saline infusion was greater than the change before saline infusion.

**Table 4. Initial Values in Experiment 3**

<table>
<thead>
<tr>
<th>Rabbit group</th>
<th>Mean arterial pressure (mm Hg)</th>
<th>Heart rate (bpm)</th>
<th>Plasma renin activity (ng ANG/ml/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline-infused donors</td>
<td>102±3</td>
<td>203±2</td>
<td>2.2±1</td>
</tr>
<tr>
<td>Normal recipients</td>
<td>97±3</td>
<td>208±2</td>
<td>2.6±2</td>
</tr>
</tbody>
</table>

Values are means ± SEM for six rabbits per group. There were no significant differences in values between the donor and recipient rabbits for either portion of the experiment.
Pressor responses to i.v. infusions of norepinephrine at 800 ng/min per kg body wt in saline-infused donor rabbits (shaded bar) and in normal recipient rabbits (clear bar) before cross-circulation of blood (X-Circ.) and in normal recipient rabbits at 5, 15, 25, and 35 minutes after cross-circulation of blood with donors. Values are means ± SEM for six rabbits in each group. ** = p < 0.01 and denotes that the pressor responses were greater in the donors than in the recipients prior to cross-circulation. *** = p < 0.01 and denotes that the pressor responses in the recipients were greater after than before cross-circulation.

Recipients averaged 0.36 ± 0.01, which represented no change.

The pressor responses to NE that was infused at 800 ng/min/kg before and at various times after the cross-circulation of blood are shown in Figures 2 and 3. As can be seen in Figure 2, the pressor responses to NE in the saline-infused donor rabbits were significantly (p < 0.01) greater than those of the normal recipient rabbits (19 ± 1 and 12 ± 1 mm Hg respectively). After the cross-circulation of blood between the saline-infused and normal recipient rabbits, the recipients had significantly greater pressor responses to NE at 5 minutes (18 ± 2 mm Hg) and at 15 minutes (17 ± 2 mm Hg) when compared to their pressor responses to NE before blood cross-circulation. The pressor responses to NE in these normal recipients at 25 minutes (14 ± 2 mm Hg) and at 35 minutes (14 ± 2 mm Hg) after blood cross-circulation, however, were not significantly different from the values seen prior to the cross-circulation. As shown in Figure 3, prior to cross-circulation the pressor responses to NE in the normal donor rabbits and in the normal recipients rabbits both averaged 12 ± 1 mm Hg, and after the cross-circulation of blood the pressor responses to NE were not significantly altered in the recipients at any of the time periods.

**Discussion**

Several studies have provided evidence suggesting that expansion of body fluid volumes will result in exaggerated pressor and vascular responses to vasoconstrictor substances. Many investigators have reported pressor and vascular hyperresponsiveness in animals treated with DOCA and given a high sodium intake (DOCA-salt hypertension). This model of hypertension has resulted in sodium retention and presumably increased body fluid volumes. Beilin and Wade observed vascular hyperresponsiveness in rats subjected to unilateral nephrectomy and placed on a high salt diet. They saw further enhancement of the hyperresponsiveness in unilaterally nephrectomized rats with a combination of a high salt intake plus treatment with DOCA.

Our present study demonstrated that saline infusion in rabbits results in pressor hyperresponsiveness to NE, as the pressor responses to each dose level of NE were significantly greater in the saline-infused than control rabbits. As shown in Experiment 2, although saline infusion resulted in a large increase in CO, the CO did not change significantly during NE infusion either before or after saline infusion. Thus, the pressor responses to NE were reflections of changes in TPR. The exaggerated increases in MAP and TPR that occurred in response to NE after saline infusion presumably were due to a more pronounced contraction of arteriolar smooth muscle cells. Therefore, these saline-infused rabbits exhibited both pressor and vascular hyperresponsiveness to NE. It is probable that the saline infusion produced expansion of body fluid volumes in these rabbits, as the saline-infused rabbits had gains in body weight and reductions in hematocrit.

Previous studies from this laboratory demonstrated that K11C rabbits with renal artery stenosis of 3 days' duration (prehypertensive rabbits) or of 30 days' duration (hypertensive rabbits) had exaggerated pressor and vascular responses to NE; this hyperresponsiveness was abolished or diminished by [Sar₁, Ile₈] ANG II, even though PRA values were normal or subnormal. Captopril, an angiotensin-converting-enzyme inhibitor, also eliminated the pressor hyperresponsiveness to NE when administered to one-kidney rabbits with 3-day renal artery stenosis and with normal PRA and plasma ANG II values. The hyperresponsiveness...
to NE in these captopril-blocked rabbits was restored by the i.v. infusion of ANG II at 2 ng/min/kg body wt, although plasma ANG II concentrations were still below the control level. Thus, in rabbits with renal artery stenosis it appears that ANG II plays an important role in pressor hyperresponsiveness to NE despite normal or subnormal plasma ANG II concentrations. Our earlier studies of a similar design showed that cross-circulation of blood between one-kidney, 3-day renal artery stenosis donor rabbits and normal recipient rabbits resulted in transfer of pressor hyperresponsiveness to NE to the normal recipients. The pressor hyperresponsiveness in the normal recipient rabbits was abolished by the administration of [Sar\(^1\), Ile\(^6\)] ANG II to the recipient rabbits immediately following the cross-circulation. Taken together, these studies in rabbits with renal artery stenosis indicated that the pressor hyperresponsiveness is mediated by a circulating hormonal factor that acts through ANG II mechanisms. Because PRA and plasma ANG II concentrations were not elevated in this model, the hormonal factor probably promoted pressor hyperresponsiveness by increasing the ANG II receptor number or affinity.

In the present study, however, [Sar\(^1\), Ile\(^6\)] ANG II failed to diminish the pressor hyperresponsiveness to NE in saline-infused rabbits, which implies that ANG II was not involved in mediating the enhanced pressor responses in these rabbits. Similarly, in earlier studies from this laboratory, 13 rabbits treated with DOCA and given a high sodium intake exhibited pressor hyperresponsiveness to NE, and these pressor responses were not altered by [Sar\(^1\), Ile\(^6\)] ANG II. Thus, the mechanisms mediating pressor hyperresponsiveness in rabbits with volume expansion probably are different from the mechanisms involved in mediating pressor hyperresponsiveness in rabbits with renal artery stenosis. It is of interest that two-kidney rabbits with unilateral renal artery stenosis of 30 days' duration had normal values for PRA, plasma volume, extracellular fluid volume, and total body water, and the pressor hyperresponsiveness to NE in these rabbits was completely abolished by [Sar\(^1\), Ile\(^6\)] ANG II. 4 On the other hand, one-kidney rabbits with 30-day renal artery stenosis had low values for PRA with increases in extracellular fluid and total body water. 14 Although [Sar\(^1\), Ile\(^6\)] ANG II diminished the pressor hyperresponsiveness to NE this ANG II analog did not abolish it. 3 Thus, the pressor hyperresponsiveness in this one-kidney renal artery stenosis model probably was due in part to the hyperresponsiveness mechanism related to renal perturbations and in part to the mechanisms associated with expansion of body fluid volumes.

A series of experiments by Haddy and co-workers 15,19 have provided insight into the mechanisms involved in the pressor and vascular hyperresponsiveness associated with body fluid volume expansion. Decreases in the ouabain-sensitive uptake of \(^{86}\)Rb were seen in blood vessels from one-kidney perinephritic hypertensive dogs, 17 from one-kidney, renal-artery-stenosed rats, 17 from rats with reduced renal mass, 17 from saline-loaded rats, 17 and from DOCA-salt hypertensive rats. 18 Decreased ouabain-sensitive uptake of rubidium is interpreted as indicating a decrease in Na\(^{+}\)-K\(^{+}\) pump activity. 15,17 Also, rats with one-kidney renal artery stenosis 19 and DOCA-salt hypertensive rats 18 were observed to have decreased Na\(^{+}\)-K\(^{+}\) ATPase activity in their myocardial membranes. It was reason that a defect in the Na\(^{+}\)-K\(^{+}\) pump in the volume-expanded animals would result in a partial depolarization of the membrane of vascular smooth muscle cells. This would make these cells more responsive. The decrease in the sodium gradient also would increase the influx of calcium 20 which also would render the vascular musculature more prone to contraction. 19

Pampani et al. 17 provided data suggesting that a plasma factor is involved in mediating the alterations in Na\(^{+}\)-K\(^{+}\) pump activity in blood vessels in one-kidney perinephritic hypertensive dogs. They found that boiled plasma from one-kidney, one clip hypertensive rats, rats with reduced renal mass and hypertension and saline-infused rats, when used to incubate tail arteries from normal rats, resulted in less ouabain-sensitive uptake of \(^{86}\)Rb when compared with normal rat tail arteries were incubated in boiled plasma from control rats. Earlier experiments by Mizukoshi and Michelakis 21 and by Michelakis et al. 22 found that when rats were injected with plasma from patients with renovascular hypertension, malignant hypertension, or essential hypertension, or with plasma from one-kidney dogs with renal hypertension, the rats had increased pressor responses to NE and ANG II. Seif et al. 23 produced hypertension in about half of the rats placed on high sodium diets, and observed that serum from hypertensive rats, when injected into assay rats, resulted in increased pressor responses to NE in these rats, whereas the injection of serum from normotensive rats had less of an effect to enhance the pressor responses to NE in the assay rats.

The present study provides direct evidence that the pressor hyperresponsiveness in animals volume-expanded by saline is mediated by a blood-borne factor, as cross-circulation of blood between saline-infused rabbits and normal rabbits resulted in pressor hyperresponsiveness in the normal animals. The transfer of hyperresponsiveness to the normal rabbits was not due to decreases in hematocrit in these animals because the hematocrit in the recipient rabbits did not change following blood cross-circulation. Also, it is unlikely that the procedures of blood cross-circulation alone could have produced disturbances that resulted in pressor hyperresponsiveness, as blood cross-circulation between pairs of normal rabbits did not alter the pressor responses in the recipient rabbits.

It has been suggested that natriuretic hormone may be the plasma factor involved in mediating the alterations in rubidium uptake and in Na\(^{+}\)-K\(^{+}\) ATPase activity and presumably in the vascular hyperresponsiveness in volume-expanded hypertensive animal models. 3,15 This hormone was implicated because plasma levels of natriuretic hormone are elevated by expansion of body fluid volumes 24 and because natriure-
natriuretic hormone suppresses sodium pump activity in the kidney\textsuperscript{10} and in the toad bladder.\textsuperscript{16} Recent studies by Plunkett et al.\textsuperscript{27} provided strong support for the participation of a natriuretic factor in vascular hyperresponsiveness. They observed that when plasma samples from saline-loaded dogs were processed by dialysis and by ion exchange chromatography, the fraction containing the natriuretic material also produced increased contractile responses to norepinephrine in third-order arterioles in a rat cremaster muscle preparation.

The findings of our present study have provided evidence that saline-infused rabbits have pressor and vascular hyperresponsiveness to NE, which is mediated by a circulating humoral factor. The pressor hyperresponsiveness in these saline-infused animals apparently does not involve ANG II. These findings are in keeping with the concept that natriuretic hormone may be involved in mediating pressor and vascular hyperresponsiveness in volume-expanded animals.

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