SUMMARY Increased pressor responses to norepinephrine and other pressor agents have been reported to occur in human essential hypertension and in several animal models of experimental hypertension. These increased responses might be related to the development of hypertension or could be a secondary consequence of the elevation in blood pressure. We have examined pressor responses to alpha-adrenoceptor agonists and to angiotensin II in male New Zealand White rabbits with perinephritic hypertension. Increased pressor responses were observed for the α₁ adrenoceptor agonist phenylephrine and the mixed α₁/α₂-adrenoceptor agonist norepinephrine but not for the α₂ adrenoceptor selective agonist guanabenz or angiotensin II. The increase occurred within 7 days of surgery and in some animals was observed when mean arterial pressure was not significantly elevated. It could not readily be attributed to intimal thickening or hypertrophy of the arterial wall, altered basal levels of norepinephrine or epinephrine, changes in norepinephrine clearance, β-adrenoceptor interactions, or decreased baroreceptor sensitivity. However, the possibility that vascular hypertrophy and decreased baroreflex sensitivity may contribute to the increase at later times cannot be excluded. In all tissues examined, specific prazosin binding was decreased in the older animals and specific clonidine binding was decreased in forebrain. However, these changes were observed in both hypertensive and sham-operated animals and were probably age-related. We believe the increased response to α₁-adrenoceptor agonists may be related to changes at a postreceptor site in the coupling of receptor activation to smooth muscle contraction. (Hypertension 5: 958–967, 1983)

KEY WORDS • alpha-adrenoceptors • sympathetic nervous system • perinephritic hypertension • catecholamines

OVER the last 30 years, there have been numerous reports of increased pressor responses to norepinephrine and other pressor agents in essential hypertension in humans. Similar observations have been made in animal models of experimental hypertension including spontaneously hypertensive rats and renal hypertensive rabbits. Changes in norepinephrine responsiveness may be mediated by β-receptors, classical postsynaptic α₁-adrenoceptors, the presynaptic regulatory α₂-adrenoceptors, or the recently proposed postsynaptic α₂-adrenoceptor which may participate in regulation of peripheral resistance. Increased responses to norepinephrine could also be the result of changes in the number of adrenoceptors or their affinity for the neurotransmitter or alternatively secondary to structural changes in the vessel wall.

Changes in α-adrenoceptor number have been observed in several brain regions from spontaneously hypertensive rats and in myocardium from renal hypertensive rats. However, in their studies, Hicks and Nahorski were unable to detect any changes in adrenoceptor number in the heart, spleen, or kidney from spontaneously hypertensive rats.

We have investigated the role of α-adrenoceptors of both α₁ and α₂ types, in the development and maintenance of experimental renal hypertension resulting from cellophane perinephritis in the rabbit.

Surgical Procedures

Male New Zealand white rabbits (2.0 to 2.5 kg) obtained from Hylyne Ltd. were anesthetized with sodium pentobarbionate (30 to 40 mg/kg). Lignocaine (2%) was infiltrated subcutaneously into both flanks and bilateral flank incisions made to expose the kidneys. The left kidney was removed, and the right kidney was wrapped in cellophane which was tied in place with silk in a figure of eight knot. Control animals were subjected to a similar procedure in that they had the left kidney removed and the right kidney mobilized and manipulated but not wrapped. After surgery, the
animals were housed in individual cages and fed a standard diet and water ad libitum. On all occasions in all phases of the study, wrapped and sham-operated animals from the same groups of littermates were studied at the same time.

Groups of animals were studied at 6–10 days, 21–28 days, 42–56 days, or 84–112 days postoperatively. All animals were between 8 and 10 weeks old at the time of surgery. Equal numbers of uninephrectomized controls and hypertensive animals were studied at each time. The numbers used for each part of the study varied from not less than 5 to not more than 10. In total, 88 hypertensive and 88 control rabbits were examined. Animals were considered to be normotensive if the resting mean arterial pressure was 100 mm Hg or less; a small number of animals from the hypertensive group (less than 10%) with a resting mean arterial pressure consistently 100 mm Hg or less 3 to 4 weeks after renal wrapping were excluded from the study.

For the cardiovascular studies, polypropylene catheters were inserted into the central artery and vein of the ear under local anesthesia (2% lignocaine). After surgery, the animals remained conscious and unrestrained and were kept in individual cages in a quiet warm laboratory throughout the study. At least 60 minutes were allowed to elapse after the minor surgical procedures before any measurements were made. Mean arterial pressure was measured with a Statham P23 1D transducer and displayed using a Grass Model 7B polygraph. Heart rate was determined from the pressure trace.

All animals were weighed prior to surgery and at weekly intervals afterward. Mean arterial pressure and heart rate were measured in all animals before pharmacological intervention, and 2 ml of blood was withdrawn from the arterial catheter for measurement of plasma norepinephrine and epinephrine by the radio metric method of Da Prada and Zurcher. A further 2 ml of blood was removed for measurement of plasma renin activity and serum urea and electrolytes from the groups of animals studied after 6–10 days and 6–8 weeks.

Assessment of Responses to Alpha-Adrenergic Agonists and Antagonists

Mean arterial pressure and heart rate were recorded and dose response curves were constructed to intravenous doses of the selective α₁-adrenoceptor agonist phenylephrine 0.5–50 μg/kg, or the mixed α₂/α₁-agonist norepinephrine 0.2–20 μg/kg. At least four doses of each agonist were given to each animal in random order. In further groups of animals, the immediate (within 15 to 30 seconds) pressor response to intravenous injection of the α₁-adrenoceptor selective agonist, guanabenz (50 and 100 μg/kg), was examined. This pressor response appears to be mediated by direct activation of peripheral postsynaptic α₁-adrenoceptors on smooth muscle of resistance vessels and has previously been used to examine altered α₁-adrenoceptor-mediated pressor responses. Although guanabenz is one of the more specific α₁-adrenoceptor agonists available, like clonidine it is a partial agonist at the α₁-adrenoceptor. Individual animals received only one dose of guanabenz in any 24-hour period, as a long-lasting central hypotensive effect with a slow offset was observed, which would have interfered with the analysis of the acute peripheral pressor response to the drug.

The effects of the selective α₁-adrenoceptor antagonist, prazosin, on mean arterial pressure, heart rate, and phenylephrine pressor responses were examined. Mean arterial pressure and heart rate were measured and phenylephrine dose response curves constructed as described above before intravenous prazosin (0.05–1.0 mg/kg). All measurements were repeated between 20 and 30 minutes after adrenoceptor blockade. At this time, prazosin-induced changes in mean arterial pressure were maximal.

Assessment of Beta-Adrenoceptor Effects and Pressor Response to Angiotensin II

Dose response curves to phenylephrine were constructed as described above in groups of control and hypertensive animals. All animals then received 2 mg/kg propranolol intravenously. In previous studies, this dose of propranolol was shown to result in a parallel displacement of both chronotropic and depressor responses to isoprenaline to the right by 50- to 100-fold. At 10 to 30 minutes after receiving propranolol, mean arterial pressure and heart rate were measured and a second dose response curve to phenylephrine constructed.

Pressor dose response curves to intravenous angiotensin II were constructed in groups of normotensive and hypertensive rabbits. At least four doses of angiotensin II (1.0–10.0 μg/kg) were given intravenously and the maximum rise in mean arterial pressure recorded.

Baroreflex Sensitivity

Baroreceptor reflex sensitivity was determined by a modification of the method of Smyth et al. Phenylephrine was infused by a Braun infusion pump through a catheter in the central vein of the ear at a rate of 0.1, 0.25, 0.5, and 1.0 mg/kg/hr, with each infusion continued for 10 minutes and the rate increased stepwise. Blood pressure and heart rate reached a plateau response within 5 minutes of commencing infusion, and measurements of mean arterial pressure and heart rate were made after 7 and 9 minutes infusion at each dose level. Baroreflex sensitivity was calculated from the slope of the linear relationship between mean arterial pressure and heart period (reciprocal of heart rate). For individual rabbits, mean arterial pressure was plotted against heart period and baroreflex sensitivity derived from the slope of line obtained by linear regression analysis.

Clearance of Intravenous Norepinephrine

After collection of a 2 ml sample of arterial blood for basal plasma norepinephrine estimation, norepinephrine was infused intravenously at infusion rates of 54
and 110 μg/kg/hr. Further blood samples (2 ml) for plasma norepinephrine estimation were collected after 12 minutes of infusion at each dose level.

Preliminary experiments revealed that an apparent steady state plasma concentration was reached within 10 minutes of commencing an intravenous infusion of norepinephrine. Clearance of norepinephrine was calculated from the rate of norepinephrine infusion and steady state plasma norepinephrine levels, as described by FitzGerald et al.\textsuperscript{17} using the formula:

\[
\text{Clearance} = \frac{\text{rate of norepinephrine infusion}}{\text{steady state norepinephrine} - \text{basal norepinephrine}}
\]

Radioligand Binding Studies

Animals were killed with sodium pentobarbitone (60 mg/kg i.v.). Tissues were removed immediately and placed on ice. Brain was divided into forebrain and hindbrain by section rostral to the corpora quadrigemina. The tissues were homogenized in 5 volumes of 0.32 M sucrose and membranes prepared by centrifugation.

Binding studies were carried out as described previously.\textsuperscript{12} [\textsuperscript{3}H]prazosin (1.5-48 nM) and [\textsuperscript{3}H]clonidine (7.5-90 nM) were used as the specific ligands, with phen tolamine 10\textsuperscript{-5} M as the displacing agent. Stereospecificity, pharmacological specificity, and kinetics of the two ligands were consistent with binding to \(\alpha_1\)- and \(\alpha_2\)-adrenoceptors, respectively. Neither guanosine 5'-triphosphate (GTP) or divalent or monovalent cations were added during incubation of the radioactive ligand with tissue membranes. Thus, under our assay conditions, clonidine should bind preferentially to the high affinity state of the receptor. The maximum number of binding sites (\(B_m\)) and their dissociation constant (\(K_d\)) were calculated by Scatchard analysis.

Drugs

We obtained [\textsuperscript{3}H]prazosin and unlabeled prazosin from Pfizer U.K. Ltd., guanabenz from Wyeth, and angiotensin II from Ciba. We purchased [\textsuperscript{3}H]clonidine from The Radiochemical Centre, Amersham, England, and all other drugs from Sigma Chemical Company, London, England.

Statistical Analysis

Results are expressed as means ± sd. In most cases, comparisons between groups were made using the nonparametric Wilcoxon test. However, to analyze phenylephrine and norepinephrine, dose response curves slopes and theoretical intercepts were calculated from the linear portion of the pressor response curve for each rabbit. The effects of hypertension, duration of hypertension, mean arterial pressure, and age on the parameters of the dose response curve were examined using linear modeling.\textsuperscript{18}

Results

Changes in Blood Pressure, Heart Rate, and Biochemical Indices

Mean arterial pressure increased above 100 mm Hg after surgery in almost all (90%) animals with one kidney removed and the contralateral kidney wrapped in cellophane. At all time intervals investigated, mean arterial pressure of the group with one wrapped kidney was significantly increased (table 1) compared to the sham-operated group. There was a small increase (\(p < 0.05\)) after 6-10 days with a further rise (\(p < 0.01\)) at the later times. During the early stages of development of hypertension variability of blood pressure was observed. In most hypertensive animals, blood pressure appeared to plateau after 3 to 6 weeks, but in a few rabbits it continued to rise over the period of study.

There was no significant difference in heart rate between the uninephrectomized controls and the animals with perinephritis hypertension at any time examined. Plasma norepinephrine, epinephrine, and renin activity were not altered in the hypertensive animals compared with controls (table 1). Even at the earliest time examined (6 to 10 days), there was no evidence of increased plasma renin activity in hypertensive animals.

<table>
<thead>
<tr>
<th>Group</th>
<th>Time (wks)</th>
<th>Weight (kg)</th>
<th>Mean arterial pressure (mm Hg)</th>
<th>Heart rate (bpm)</th>
<th>Plasma norepinephrine (nM)</th>
<th>Plasma epinephrine (nM)</th>
<th>Plasma renin activity (nM angiotensin II generated/ml plasma/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHT</td>
<td>1-1½</td>
<td>2.2 ± 0.3</td>
<td>105 ± 10*</td>
<td>237 ± 54</td>
<td>4.3 ± 2.3</td>
<td>1.3 ± 1.0</td>
<td>2.8 ± 1.9</td>
</tr>
<tr>
<td>NT</td>
<td>2.4 ± 0.4</td>
<td>80 ± 8</td>
<td>203 ± 20</td>
<td>4.6 ± 2.5</td>
<td>1.6 ± 0.9</td>
<td>3.2 ± 1.2</td>
<td>-</td>
</tr>
<tr>
<td>PHT</td>
<td>3-4</td>
<td>2.2 ± 0.3</td>
<td>129 ± 17†</td>
<td>225 ± 25</td>
<td>3.1 ± 1.0</td>
<td>1.3 ± 0.4</td>
<td>-</td>
</tr>
<tr>
<td>NT</td>
<td>2.4 ± 0.4</td>
<td>81 ± 9</td>
<td>200 ± 25</td>
<td>3.5 ± 1.2</td>
<td>1.6 ± 0.2</td>
<td>3.2 ± 1.2</td>
<td>-</td>
</tr>
<tr>
<td>PHT</td>
<td>6-8</td>
<td>2.5 ± 0.4</td>
<td>139 ± 16†</td>
<td>234 ± 33</td>
<td>3.1 ± 1.3</td>
<td>1.5 ± 0.2</td>
<td>2.6 ± 2.5</td>
</tr>
<tr>
<td>NT</td>
<td>2.6 ± 0.3</td>
<td>91 ± 16</td>
<td>209 ± 31</td>
<td>3.5 ± 1.9</td>
<td>1.5 ± 0.1</td>
<td>3.6 ± 2.0</td>
<td>-</td>
</tr>
<tr>
<td>PHT</td>
<td>12-16</td>
<td>3.0 ± 0.6</td>
<td>124 ± 13†</td>
<td>240 ± 20</td>
<td>5.7 ± 2.5</td>
<td>1.7 ± 0.6</td>
<td>-</td>
</tr>
<tr>
<td>NT</td>
<td>2.9 ± 0.8</td>
<td>88 ± 10</td>
<td>223 ± 62</td>
<td>6.3 ± 2.9</td>
<td>1.7 ± 0.7</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

\*\textsuperscript{p} < 0.05 and \#\textsuperscript{p} < 0.01 when animals with perinephritis hypertension were compared with uninephrectomized controls that had been operated at the same time. Wilcoxon nonparametric test used for comparisons. Results expressed as means ± sd. n = 8-12 for each group.
All animals resumed eating and drinking within 24 hours of renal surgery. Several animals lost 0.1–0.2 kg in the first few postoperative days, and there was little weight gain by either group in the first 3 weeks after surgery. After this, both groups of animals gained weight at the same rate, so that the weight of the hypertensive and control rabbits was similar at all times of study (table 1). Neither removal of one kidney nor wrapping of the contralateral kidney in cellophane resulted in any changes in plasma levels of sodium, potassium, chloride, or bicarbonate in either the short-term (6 to 10 days) or longer term (6 to 8 weeks) postoperatively. There was no gross impairment of renal excretory function, as plasma urea or creatinine were not altered.

Pressor Responses to Alpha-Adrenoceptor Agonists and Angiotensin II

Intravenous phenylephrine and norepinephrine caused dose-related increases in mean arterial pressure in both the uninephrectomized control animals and in the rabbits with perinephritic hypertension. The absolute pressor response to a given dose of either phenylephrine or norepinephrine was consistently greater in the hypertensive rabbits (fig. 1). However when the data were plotted as a percentage change no significant difference between the groups was observed (fig. 2). The increased pressor responses to phenylephrine and norepinephrine in the hypertensive animals were manifested as a parallel shift to the left in the pressor log dose response curves. There was no significant difference between the slopes of the linear portion of the pressor dose response curves from normotensive and hypertensive rabbits. Linear modeling showed that the increased pressor response to phenylephrine and norepinephrine was statistically significant at all times studied, from 6–10 days to 3–4 months postoperatively. This increased response to phenylephrine and norepinephrine was independent of the absolute level of mean arterial pressure and the duration of hypertension in the animals with perinephritic hypertension.

In contrast to responses to phenylephrine and norepinephrine, there were no differences in pressor responses to the α₁-adrenoceptor selective agonist, guanabenz, between uninephrectomized controls and animals with perinephritic hypertension at any of the times studied (table 2). In the animals with perinephritic hypertension, the mean pressor response to guanabenz (100 µg/kg) ranged from 23 ± 2 mm Hg at 6–10 days postoperatively to 31 ± 5 mm Hg at 6–8 weeks after surgery; in the uninephrectomized controls, the pressor response ranged from 20 ± 5 mm Hg 3 to 4 weeks postoperatively to 26 ± 10 mm Hg 6–8 weeks postoperatively.

In addition, there was no significant difference between pressor dose response curves to angiotensin II in groups of hypertensive animals and groups of uninephrectomized controls either 7 days or 6 weeks postoperatively (fig. 3). Thus, while no change was observed in pressor responses to angiotensin II and the α₁-adrenoceptor agonist, there was an increase in the pressor response to phenylephrine and norepinephrine.

**Effect of α₁- and β-Adrenoceptor Antagonism**

Prazosin 0.05–1.0 mg/kg administered intravenously caused similar dose-related falls in mean arterial pressure in the uninephrectomized control animals and in those with perinephritic hypertension at all times examined. After the highest dose of prazosin (1.0 mg/kg), mean arterial pressure fell by 24 ± 9, 24 ± 9, and 20 ± 11 mm Hg in the hypertensive animals studied 6–10 days, 3–4 weeks, and 3–4 months postoperatively, and by 26 ± 10, 20 ± 10, and 25 ± 7 mm Hg in the normotensive controls (table 3). There was an increase in heart rate in all animals after administration of prazosin; however, this tachycardia could not be related to the dose of prazosin, fall in blood pressure, or basal mean arterial pressure. The degree of postsynaptic α₁-adrenoceptor blockade, measured by the shift to the right in phenylephrine pressor dose response curves (phenylephrine dose ratio), was dose-related and was similar at each dose level in normotensive and hypertensive animals (table 3).

The effects of β-adrenoceptor antagonism were only studied in animals with established hypertension 3 to 4 months after renal wrapping. Beta-adrenoceptor blockade with intravenous propranolol (2 mg/kg) caused a similar decrease in both mean arterial pressure and heart rate in animals with perinephritic hypertension and in sham-operated controls. Mean arterial pressure fell by 5 ± 2 and 8 ± 2 mm Hg in the two groups respectively and heart rate by 11 ± 10 and 9 ± 10 bpm. Beta-adrenoceptor blockade had no significant effect on phenylephrine responses in either group.

**Baroreflex Sensitivity**

Baroreflex sensitivity was similar in the animals with perinephritic hypertension and in the uninephrectomized controls, 7.4 ± 4.2 and 6.2 ± 3.2 msec/mm Hg, at the earliest time examined (6–10 days postoperatively) although at this time mean arterial pressure was already significantly raised in the perinephritis group (table 1). In established hypertension there was a
consistent decrease in baroreflex sensitivity in the hypertensive animals as revealed by a reduction in the slope of the regression line relating arterial pressure and heart period. The decrease had reached a maximum by 4 weeks postoperatively being 2.8 ± 1.0 msec/mm Hg at this time and 2.8 ± 1.9 msec/mm Hg 3–4 months after surgery compared to 9.7 ± 3.9 and 9.4 ± 4.3 msec/mm Hg at these times in the control animals (table 4).

There was no significant relationship between the decrease in baroreflex sensitivity and level of mean arterial pressure in the animals with established hyper-

![Figure 1](http://hyper.ahajournals.org/)

**Figure 1.** Pressor dose response curves to i.v. injections of phenylephrine and norepinephrine in conscious rabbits showing absolute changes in mean arterial pressure. Animals with perinephritis hypertension (○–○); uninephrectomized controls (●–●). All points shown as means ± sD. n = 6–15.

A. Phenylephrine pressor response 6–10 days postoperatively.
B. Phenylephrine pressor response 3–4 weeks postoperatively.
C. Phenylephrine pressor response 6–8 weeks postoperatively.
D. Phenylephrine pressor response 3–4 months postoperatively.
E. Norepinephrine pressor response 6–10 days postoperatively.
F. Norepinephrine pressor response 3–4 weeks postoperatively.
G. Norepinephrine pressor response 6–8 weeks postoperatively.
H. Norepinephrine pressor response 3–4 months postoperatively.
ALPHA-ADRENOCEPTORS AND PERINEPHRITIS HYPERTENSION/Hamilton and Reid 963

**Figure 2.** Pressor dose response curves to i.v. injections of phenylephrine and norepinephrine showing percentage change in mean arterial pressure. Animals with perinephritis hypertension (○—○); uninephrectomized controls (●—●). All points shown as mean ± so; n = 6–15. A. Phenylephrine pressor response 3–4 weeks postoperatively. B. Norepinephrine pressor response 3–4 weeks postoperatively.

**Figure 3.** Pressor dose response curves to angiotensin II. All points shown as means ± so, n = 6. A = animals studied 7–10 days postoperatively. B = animals studied 6–8 weeks postoperatively. Uninephrectomized controls (●—●); perinephritis hypertensives (○—○).

**Table 3.** Effects of Prazosin on Mean Arterial Pressure, Heart Rate and Response to Phenylephrine

<table>
<thead>
<tr>
<th>Prazosin (mg/kg)</th>
<th>Fall in MAP (mm Hg)</th>
<th>Increase in heart rate (bpm)</th>
<th>Phenylephrine dose ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PHT</td>
<td>NT</td>
<td>PHT</td>
</tr>
<tr>
<td>6 to 10 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>15 ± 10</td>
<td>14 ± 11</td>
<td>32 ± 15</td>
</tr>
<tr>
<td>0.5</td>
<td>21 ± 14</td>
<td>21 ± 12</td>
<td>56 ± 39</td>
</tr>
<tr>
<td>1.0</td>
<td>24 ± 9</td>
<td>26 ± 10</td>
<td>34 ± 54</td>
</tr>
<tr>
<td>3 to 4 weeks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>15 ± 10</td>
<td>14 ± 7</td>
<td>60 ± 50</td>
</tr>
<tr>
<td>0.1</td>
<td>21 ± 10</td>
<td>16 ± 8</td>
<td>72 ± 55</td>
</tr>
<tr>
<td>0.5</td>
<td>24 ± 12</td>
<td>20 ± 11</td>
<td>84 ± 34</td>
</tr>
<tr>
<td>1.0</td>
<td>34 ± 9</td>
<td>25 ± 7</td>
<td>73 ± 64</td>
</tr>
<tr>
<td>3 to 4 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>13 ± 8</td>
<td>15 ± 6</td>
<td>50 ± 23</td>
</tr>
<tr>
<td>0.1</td>
<td>19 ± 7</td>
<td>18 ± 6</td>
<td>64 ± 42</td>
</tr>
<tr>
<td>0.5</td>
<td>19 ± 3</td>
<td>11 ± 9</td>
<td>70 ± 35</td>
</tr>
<tr>
<td>1.0</td>
<td>20 ± 11</td>
<td>20 ± 10</td>
<td>63 ± 44</td>
</tr>
</tbody>
</table>

Results are expressed as means ± so. At least five observations were made for each dose of prazosin. PHT = perinephritis hypertensive animals; NT = uninephrectomized normotensive controls; MAP = mean blood pressure. No significant differences were observed when normotensive and hypertensive animals compared using the nonparametric Wilcoxon test.
TABLE 4. Baroreflex Sensitivity During the Development of Perinephritis Hypertension.

<table>
<thead>
<tr>
<th>Time</th>
<th>Uninephrectomised controls</th>
<th>Perinephritis hypertensives</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean arterial pressure (mm Hg)</td>
<td>Mean arterial pressure (mm Hg)</td>
</tr>
<tr>
<td>7-10 days</td>
<td>66 ± 6</td>
<td>98 ± 3</td>
</tr>
<tr>
<td>13-15 days</td>
<td>72 ± 8</td>
<td>117 ± 10</td>
</tr>
<tr>
<td>3-4 wks</td>
<td>80 ± 10</td>
<td>119 ± 11</td>
</tr>
<tr>
<td>3-4 mos</td>
<td>73 ± 4</td>
<td>125 ± 11</td>
</tr>
</tbody>
</table>

Uninephrectomized controls and perinephritis hypertensives compared using the nonparametric Wilcoxon test, n = 5 to 7 in each group at each time.

TABLE 5. Norepinephrine Clearance in Rabbits with Perinephritis Hypertension and in Unnephrectomized Control Animals

<table>
<thead>
<tr>
<th>Time postoperative</th>
<th>Clearance (1 hr⁻¹ kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>54 µg/kg/hr infusion</td>
</tr>
<tr>
<td>3-4 wks</td>
<td>3.8 ± 3.5</td>
</tr>
<tr>
<td>6-8 wks</td>
<td>3.8 ± 2.0</td>
</tr>
<tr>
<td>3-4 mos</td>
<td>3.3 ± 1.5</td>
</tr>
</tbody>
</table>

NT = normotensive unnephrectomized controls; PHT = perinephritic hypertensive animals; n = 5-7. Results expressed as means ± SD.

No significant differences in Bmax or Kd were observed when normotensive animals were compared with hypertensive rabbits n = 5-8 for each tissue in each group. Bmax is expressed as fmolcs/mg protein, Kd as nM.

Norepinephrine Clearance

Clearance of intravenously infused norepinephrine was not altered in hypertensive animals when compared to clearance in the normotensive controls at any of the times studied. In the normotensive uninephrectomized controls, norepinephrine clearance ranged from 3.3 ± 1.5 to 4.3 ± 2.5 liters/hr/kg, and in the animals with perinephritis hypertension, from 3.0 ± 1.8 to 4.2 ± 3.0 liters/hr/kg (table 5).

Radioligand Binding Studies

No difference was observed in the maximum number of prazosin or clonidine binding sites or their dissociation constants in spleen, heart, forebrain, or hindbrain, between uninephrectomized controls and animals with perinephritic hypertension either 6-8 weeks or 3-4 months after surgery. However, in both the normotensive and hypertensive animals, there was a significant decrease in the number of prazosin binding sites in all tissues examined at 3-4 months postoperatively compared to 6-8 weeks postoperatively (table 6) and also a decrease in clonidine binding in the forebrain, suggesting that age-dependent changes in alpha-receptor binding sites might occur. These changes could not be related to differences in recovery of membrane protein.

Discussion

Perinephritic hypertension in the rabbit has been used to study hemodynamic and biochemical changes associated with the development of raised blood pres-
sure. This model of hypertension is not associated with significant renal impairment, and in the present study no plasma electrolyte abnormalities were noted. Since urinary excretion of electrolytes was not determined, however, increased sodium retention in the hypertensive cannot be excluded. At times from 1 week onward, there were no changes in plasma renin activity or plasma norepinephrine or epinephrine. Thus, neither increased sympathoneural nor sympathoadrenal activity underlay the raised pressure although it has previously been shown that destruction of central noradrenergic neurons with intracisternal 6-hydroxydopamine can prevent the rise in blood pressure after renal wrapping.19 The lack of change in plasma renin activity implies that the renin-angiotensin system was not primarily involved in the genesis of hypertension in this model. There was, however, an increase in pressor responses to both intravenous doses and to infusions of the \( \alpha_1 \)-adrenoceptor agonist, phenylephrine, and to the endogenous transmitter, norepinephrine, which activates both \( \alpha_1 \) and \( \alpha_2 \)-adrenoceptors when absolute changes in mean arterial pressure were plotted.

However, when the data were expressed as a percentage change, this difference was not apparent; this observation does not explain the differences between enhanced phenylephrine and norepinephrine responses and unchanged angiotensin II and guanabenz responses. Indeed, we are uncertain of the relative validity of expressing pressor responses in absolute terms or as a percentage. In established perinephritic hypertension in the rabbit, cardiac output may be decreased20-21 and peripheral resistance increased. The increased pressor response to \( \alpha_1 \)-adrenoceptor agonists observed in the hypertensive rabbits is unlikely to be the result of a generalized increase in response to circulating pressor agents, since no increases in pressor responses to the \( \alpha_1 \)-adrenoceptor agonist, guanabenz, or to angiotensin II were observed in this study.

In similar studies, Korner5 observed greater rises in hindlimb vascular resistance in rabbits with bilateral renal-cellophane-wrap hypertension than in normotensive rabbits. Thus, although pressor responses leading to smooth muscle contraction may be increased in preparations from small blood vessels in the rabbit.

An alteration in \( \alpha_1 \)-adrenoceptor number or sensitivity could underlie the increased phenylephrine response. However, radioligand-binding studies with \( [\text{H}] \) prazosin did not show any change in the maximum number of prazosin-binding sites or their dissociation constants, at least in the heart, spleen, forebrain, or hindbrain in hypertensive animals compared to controls. Hicks and Nahorski3 were also unable to find any changes in \( [\text{H}] \) prazosin binding to membranes from spleen, ventricle, and kidney of spontaneously hypertensive rats, although in renal hypertensive Goldblatt rats, Woodcock and Johnson10 using the non-specific ligand \( [\text{H}] \) dihydroergocryptine, which binds to both \( \alpha_1 \) and \( \alpha_2 \)-adrenoceptors, found a decrease in adrenoceptor concentration. Although no change in \( [\text{H}] \) prazosin binding was observed in our study, the possibility that the number of receptors was increased in arterioles and small resistance vessels cannot be ruled out. Changes in receptor number are not necessarily a generalized phenomenon and localized changes in adrenoceptor number in specific brain regions have been reported.9 Unfortunately, although others have reported binding studies in vascular membranes from the rat22 and dog,23 we have been unable to determine specific \( \alpha_1 \)-adrenoceptor binding with \( [\text{H}] \) prazosin or clonidine in preparations from small blood vessels in the rabbit.

Although no hypertension-related change in \( \alpha_1 \)-adrenoceptor binding was observed, specific prazosin binding was decreased in all tissues examined in both hypertensive and normotensive animals from the older group. These animals would be 24-30 weeks old compared to 10-14 weeks in the case of the younger group. In contrast, specific clonidine binding was only reduced in the forebrain membranes of the older animals. We believe these age-related changes are unaffected by hypertension, but this observation does highlight differences in the regulation of the two types of \( \alpha_1 \)-adrenoceptor.

There is further indirect evidence against a change in \( \alpha_1 \)-adrenoceptor number in the hypertensive animals. This comes from the studies with the selective \( \alpha_1 \)-adrenoceptor antagonist, prazosin. Prazosin caused a similar degree of \( \alpha_1 \)-adrenoceptor blockade (as measured by the phenylephrine dose ratio) and similar falls in mean arterial pressure in the normotensive and hypertensive animals. Thus, although pressor responses to the \( \alpha_1 \)-adrenoceptor agonist were increased, responses to the antagonist were not altered. These findings suggest that the \( \alpha_1 \)-adrenoceptor agonist itself is not modified in perinephritic hypertension. Thus, the agonist will activate the receptor, initiating a change of postreceptor responses leading to smooth muscle contraction, while the antagonist will simply block the receptor. The increased response to \( \alpha_1 \)-adrenoceptor agonists could be a secondary consequence of other changes or could be a result of altered post receptor or postbinding site responses in the hypertensive animals.

There have been several reports of changes in \( \beta \)-adrenoceptor number in experimental hypertension,10,26 but \( \beta \)-adrenoceptor blockade with proprano-
lool had no effect on phenylephrine pressor responses in either the hypertensive animals or the uninephrectomized controls, making it very unlikely that the increased pressor response was a secondary consequence of changes in β-adrenoceptors in the hypertensive animals.

The studies in clearance of infused norepinephrine excluded the possibility that the increased response to norepinephrine was a result of reduced clearance of the amine. These studies also suggest that changes in clearance did not confound the interpretation of plasma norepinephrine levels. Reduced neuronal uptake (uptake,) is unlikely as phenylephrine, which is a poor substrate for neuronal uptake, showed similar increases in responsiveness.

Baroreflex sensitivity was decreased in the hypertensive animals and the attenuation of the buffering by bradycardia of the rises in mean arterial pressure could have contributed to the increased pressor response. However, no significant decrease in baroreflex sensitivity was observed in the animals examined 6–10 days postoperatively, although the pressor response to phenylephrine was increased at that time. Moreover, in animals that underwent sinoaortic denervation to achieve complete baroreceptor deafferentation, although the bradycardia following phenylephrine injection was abolished, the shift to the left in the phenylephrine dose response curve was small (phenylephrine/dose ratio = 1.7). Thus, we believe that the relatively small decrease in baroreflex sensitivity is unlikely to be a major factor in causing increased phenylephrine pressor response in the hypertensive animals, although a small contribution cannot be excluded.

We can only speculate on the nature of the possible mechanisms of enhanced pressor responses and of the trigger that leads to vascular changes even before hypertension has developed fully. We believe that our data would support a postreceptor mechanism. In spontaneously hypertensive rats, changes have been described in vascular responses that develop early, alter the electrophysiological properties of smooth muscle, and may be the parallel of our observations in the rabbit. These mechanisms may involve changes in transport of sodium and other ions across cell membrane, including those of vascular smooth muscle cells. Moreover, vascular α1- and α2-adrenoceptors appear to elicit vasoconstriction by different mechanisms. Alpha1-adrenoceptors, but not α2-adrenoceptors, appear to be coupled in an inhibitory fashion to the adenylate cyclase in vascular smooth muscle cells, and α2-adrenoceptors are probably coupled to calcium flux by different mechanisms to those coupling α1-adrenoceptors to calcium flux. Thus, altered postreceptor mechanisms in perinephric hypertension could well affect pressor responses to α1 but not α2 adrenoceptor agonists.

In summary, an increased absolute pressor response to α1- but not α2-adrenoceptor agonists was observed in rabbits with perinephric hypertension. This increase could not be readily attributed to intimal thickening or hypertrophy of the arterial wall, altered basal levels of norepinephrine or epinephrine, changes in norepinephrine clearance, adrenoceptor interactions, decreased baroreceptor sensitivity, or a change in α1-adrenoceptor binding characteristics. The change in α1-adrenoceptor responsiveness was not generalized and not observed after angiotensin II. We would propose that the increased response may reflect changes at a postreceptor site in the coupling of alpha-receptor activation to vascular contraction.

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