Pathogenesis of Hypertension in Rats with Chronic Aortic Baroreceptor Deafferentation

GREGORY D. FINK, PH.D., FIDELMA KENNEDY, B.S., WILLIAM J. BRYAN, M.S., AND ANDREW WERBER, B.S.

SUMMARY In an attempt to produce a form of chronic neurogenic hypertension without the increased blood pressure lability which is characteristic of total baroreceptor removal, selective aortic baroreceptor deafferentation (ABD) was performed in rats. Blood pressure, blood pressure variability, heart rate, plasma and extracellular fluid volumes, and the effect of total autonomic blockade were determined in male rats 1 month following ABD. Rats with ABD had significantly higher systolic, diastolic, and mean arterial blood pressures than did sham-operated animals, but the standard deviation of pressure measured repetitively over a 1-hour period was not significantly greater. Total autonomic blockade with atropine, propranolol, and phenotolamine lowered blood pressure and heart rate to a similar level in ABD and sham-operated rats. Extracellular fluid volume was not different in the two groups of rats, but plasma volume was significantly lower in rats with ABD. Despite the overall reduction in plasma volume, there was a significant positive correlation between plasma volume and blood pressure in ABD rats; no such correlation was observed in sham-operated rats. It was concluded that ABD produces a mild, chronic hypertension in rats without marked pressure lability. Although the hypertension appears to be "neurogenic" in that it is abolished by autonomic blockade, volume factors also may contribute to the increased blood pressure. (Hypertension 2: 319-325, 1980)

KEY WORDS • hypertension • autonomic nervous system • baroreceptors • plasma volume

THE existence of a true "neurogenic" hypertension produced by attenuation of the high-pressure baroreflex arc is a matter of controversy. Although many early studies described a chronic hypertension following deafferentation of the carotid and aortic baroreceptors1-4 (referred to here as total baroreceptor denervation), the method used to measure arterial pressure may have been too stressful to allow accurate pressure determination. Recent studies4-5 using more sophisticated techniques of pressure measurement and data analysis indicated that total baroreceptor denervation greatly increases minute-to-minute blood pressure variability (lability), but only slightly increases the average blood pressure level. Other recent studies6-8 using similar but not identical techniques, however, have shown that total baroreceptor removal (peripheral or central) will indeed produce a sustained arterial hypertension, albeit with increased pressure lability. In general, studies utilizing continuous (24 hr/day) pressure measurements4-8 have failed to document an increased arterial pressure following total baroreceptor denervation, whereas those utilizing careful measurement at a fixed time of day have found elevated pressures. Clearly, the minute-to-minute and circadian variability of arterial pressure after total baroreceptor denervation is a complicating factor in the interpretation of such experiments.

A few investigators have examined the effects on arterial pressure of selective baroreceptor removal. Selective baroreceptor attenuation would presumably involve less pressure lability, since some baroreflex activity would remain intact. Krieger4 reported a marked hypertension in two rats subjected to aortic baroreceptor deafferentation. Pressure lability was not measured in these animals. Selective carotid sinus baroreceptor deafferentation produced a less marked effect in Krieger's studies. Scher and Ito8-10 reported that aortic baroreceptor deafferentation in the dog caused a persistent hypertension without increased pressure lability; carotid sinus deafferentation did not produce chronic hypertension or pressure lability.10 On the other hand, McRitchie et al.4 found that selective carotid sinus deafferentation in the dog caused a moderate hypertension for up to 1 month; these authors did not quantify pressure variability.

Surprisingly, few attempts have been made to document the cause of hypertension in conscious animals following total or partial baroreceptor denervation.
Although increased biochemical indications of sympathetic activity were observed in rabbits and rats following chronic total baroreceptor denervation,\textsuperscript{11, 13} no evidence was provided to indicate that this activity was translated into neurogenic vasoconstriction or increased cardiac output. Very recently, however, it was demonstrated that sympathoinhibition with clonidine would normalize arterial pressure in dogs with chronic hypertension produced either by lesions of the nucleus tractus solitarius or total peripheral (sino-aortic) baroreceptor denervation.\textsuperscript{13}

The very possibility of chronic “neurogenic” hypertension has been questioned on theoretical grounds by Guyton and associates.\textsuperscript{14} These workers maintain that any rise in arterial pressure, unaccompanied by alterations in renal function, will result in increased renal fluid loss and body fluid volume contraction, and hence a return of arterial pressure to levels very near normal. To our knowledge, such a sequence of events heretofore has not been documented in neurogenic hypertension, although independent effects of total or partial baroreceptor denervation on renal function could complicate interpretation of such experiments. Recent studies from this laboratory,\textsuperscript{15} however, indicated that aortic baroreceptor deafferentation (ABD) in rats produced an immediate rapid rise in arterial pressure, followed by a significant contraction of body fluid volumes, consistent with the predictions of the systems model for circulatory control proposed by Guyton and colleagues.\textsuperscript{14} The purposes of the present investigation were to determine: 1) does ABD cause a sustained “neurogenic” hypertension in rats; 2) is blood pressure more labile in rats with chronic ABD; and 3) what effect does chronic ABD have on body fluid volumes and their relationship to arterial pressure?

Methods

Male Sprague-Dawley rats from Spartan Farms (Haslett, MI) were used in these experiments. Animals weighed 200–250 g at the beginning of the study. They were housed two to three per cage in air-conditioned, light-cycled quarters, and given normal rat chow (Wayne Lab-Blox) and tap water ad libitum. Following placement of indwelling catheters, the rats were housed individually to protect the protruding catheter ends.

The ABD was performed according to the method of Krieger.\textsuperscript{4} Anesthesia was produced by i.p. injection of sodium pentobarbital (50 mg/kg). Atropine (1 mg/kg, i.p.) also was administered to minimize pulmonary congestion. Through a ventral neck incision, the superior laryngeal, cervical sympathetic, and aortic depressor nerves (where present) were sectioned bilaterally. Sham operation consisted of isolating nerves without sectioning. A single postoperative injection of procaine penicillin G (20,000 U) and dihydrostreptomycin (25 mg) was given to each rat. The criterion for “successful” ABD in all but the final series of rats in this study was an accentuated (in magnitude and duration) carotid occlusion pressor reflex under pentobarbital anesthesia (50 mg/kg, i.p.) compared to that of normal rats.

One month following ABD, under sodium pentobarbital anesthesia (50 mg/kg, i.p.), tapered polyethylene catheters (PE-50) were placed in the abdominal aorta and vena cava, via a femoral artery and vein respectively. In an initial study on 16 rats (8 sham-operated, 8 ABD), the animals were allowed to recover for 2–3 days. Catheters were filled with heparinized saline solution (50 U/ml) and flushed daily. Pressure recording was carried out in the rat's home cage. The arterial catheter was connected to a pressure transducer (Statham P23Db or Ailtech MS-10) via long flexible connecting tubing (PE-50). Blood pressure was recorded continuously on a Grass polygraph. All recordings were carried out in a quiet, lighted room between 8 and 12 a.m. After the rat's initial exploratory movements had ceased (15–30 minutes), a 1-hour recording session was begun. Systolic, diastolic, and mean (systolic + 2 X diastolic/3) arterial pressure were noted at exactly 5-minute intervals throughout the hour (12 measurements). During this hour, the behavior of the rats ranged from quiet sitting to grooming behavior. Eating or drinking were not allowed. Average pressures were calculated from the 12 interval measurements, and the standard deviation of these pressures for each rat was calculated by the usual formula.

In a second group of 30 rats (15 sham-operated, 15 ABD), arterial pressure, heart rate, fluid volumes, and response to total autonomic blockade were assessed 1 month after ABD or sham operation. Total autonomic blockade is here defined as combined administration of supramaximal doses of drugs known to competitively block the muscarinic receptors of the parasympathetic neuroeffector junction (atropine), the beta receptors of the sympathetic neuroeffector junction (propranolol), and alpha receptors of the sympathetic neuroeffector junction (phentolamine). This combination leaves the entire animal without functional autonomic effectors. Since the heart is invested with only two types of functionally important autonomic receptor types — muscarinic receptors mediating vagal responses and beta-adrenergic receptors mediating sympathetic nerve responses — total cardiac autonomic blockade can be achieved with the combination of atropine and propranolol alone, leaving vascular sympathetic neuroeffectors (alpha-receptor mediated) intact. One or 2 days after placement of indwelling arterial and venous catheters as previously described, arterial blood pressure was recorded as before, except that only a single reading was recorded after 15–30 minutes of equilibration. Heart rate was counted directly from the blood pressure tracing. A 0.6 ml control blood sample was then drawn through the arterial catheter, and 0.2 ml of a solution containing 5 mg Evans blue dye and 50 mg of sodium thiocyanate per ml was injected via the venous catheter. Additional 0.6 ml arterial blood samples were drawn at 10 and 30 minutes following tracer injection. Evans blue and thiocyanate concentrations in the plasma samples were determined spec-
trophotometrically according to standard procedures. Plasma volume and extracellular fluid volume were estimated from the concentration of Evans blue in the 10-minute plasma sample and of thiocyanate in the 30-minute plasma sample respectively. The following day, arterial pressure and heart rate were again measured, and the average of this and the previous day's reading was recorded for each animal. Atropine (1 mg/kg), propranolol (1 mg/kg), and phentolamine (2 mg/kg) were then administered intravenously in sequential order at 5-10-minute intervals, and arterial pressure and heart rate were redetermined at least 5 minutes after each drug injection.

In a final group of 19 rats (11 sham-operated, 8 ABD), baroreflex activity was assessed 1-2 days following placement of indwelling arterial and venous catheters (1 month following sham operation or ABD). Arterial pressure in the conscious rats was raised in steps by infusion of phenylephrine (10-200 μg/kg/min), then lowered by infusion of nitroglycerin (50-200 μg/kg/min) alone or in combination with phentolamine (0.2-0.5 mg/kg). Changes in heart rate during steady-state blood pressure alterations were counted directly off the pressure record, or measured continuously using a cardiotachometer (Grass 7P4C) triggered by the pressure pulses.

Data were statistically analyzed using Student's t test, analysis of variance, and standard linear regression and correlation methods. Probability levels of less than 0.05 were considered significant.

Results

Table 1 illustrates arterial pressure level and variability in a group of rats 1 month following ABD or sham operation. Systolic, diastolic, and mean arterial pressure were significantly greater in ABD rats than in control animals. The variability of these pressure readings over a 1-hour period (standard deviation), however, was not significantly different between the two groups.

Mean arterial pressures and heart rates of another group of 15 sham operated and 15 ABD rats are shown in figure 1. Average mean arterial pressure in the ABD rats (134 ± 4 mm Hg) was significantly higher than that of sham-operated rats (120 ± 2 mm Hg). Although all animals with ABD did not have arterial pressures outside the range of the normal rats, note that eight of 15 rats with ABD had pressures greater than the highest values seen in control rats. Average heart rate in ABD rats (419 ± 7 beats/min) was also significantly higher than in the sham-operated rats (384 ± 9 beats/min). Again note, however, that some rats with ABD had heart rates within the normal range. There was no significant correlation between heart rate and arterial pressure in either group of rats. Figure 2 shows the effect of total autonomic blockade on arterial pressure in this group of rats. Total cardiac autonomic blockade (atropine + propranolol) did not significantly lower arterial pressure in either group of rats. The slight fall in arterial pressure following administration of atropine

<table>
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<th>Animals</th>
<th>Systolic</th>
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<tr>
<td>Sham-operated</td>
<td>163 ± 3</td>
<td>129 ± 3</td>
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<tr>
<td>ABD</td>
<td>178 ± 5*</td>
<td>145 ± 3*</td>
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<td>SD of AP</td>
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<td>Sham-operated</td>
<td>8 ± 1</td>
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<tr>
<td>ABD</td>
<td>9 ± 1</td>
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SD = standard deviation; AP = arterial pressure.

*Difference significant at p < 0.05.
to ABD rats probably was the result of the weak ganglion-blocking properties of this drug. Arterial pressure would generally return to preblock levels even if propranolol was not given, but the return of pressure following propranolol injection also could possibly reflect antagonism of epinephrine-induced, beta-receptor mediated muscle vasodilation. Following the administration of all three autonomic blockers, mean arterial pressure was the same in ABD (105 ± 3 mm Hg) and sham-operated rats (107 ± 3 mm Hg). The decrease in arterial pressure during autonomic blockade was significantly greater in ABD rats (29 ± 3 mm Hg, 22%) than in sham-operated controls (13 ± 2 mm Hg, 11%). Figure 3 shows that atropine treatment increased heart rate to the same level in ABD (470 ± 12 beats/min) and sham-operated rats (471 ± 10 beats/min). Further treatment with propranolol and phentolamine decreased heart rate identically in the two groups of animals.

Plasma and extracellular fluid volumes of sham-operated and ABD rats 1 month following operation are shown in figure 4. Plasma volume in ABD rats (3.02 ± 0.28 ml/100 g) was significantly less than that of sham-operated rats (3.91 ± 0.30 ml/100 g), but extracellular fluid volume was similar in the two groups (sham-operated, 27.0 ± 2.4 ml/100 g; ABD, 25.9 ± 2.0 ml/100 g). Figure 5 illustrates the correlation between resting mean arterial pressure and plasma volume in these rats. Mean arterial pressure exhibited a significant (p < 0.01) positive correlation (r = 0.74) with plasma volume in ABD rats, but only a slight positive correlation (r = 0.33) in control rats. The slope of the regression of arterial pressure on plasma volume was significantly (p < 0.05) greater in ABD rats than in sham-operated rats. The individual regression of arterial pressure on plasma volume was highly significant in ABD rats (p < 0.01) but was not different from zero in sham-operated rats.

Finally, figure 6 demonstrates the marked attenuation of overall baroreflex regulation of heart rate in rats with ABD. The gain of the reflex was significantly lower in ABD rats (0.40 ± 0.06 msec/mm Hg) than in sham-operated rats (1.50 ± 0.10 msec/mm Hg), and the overall baroreflex range (HP [saturation

**Figure 3.** Changes in heart rate during consecutive intravenous administration of atropine (ATR), propranolol (PROP), and phentolamine (PHENT) in conscious rats 1 month following aortic baroreceptor deafferentation (ABD) or sham operation. C = control measurements. Points represent mean ± SEM. The asterisk indicates a significant difference (p < 0.05) between groups.

**Figure 4.** Plasma volume and extracellular fluid volume in conscious rats 1 month following aortic baroreceptor deafferentation (ABD) or sham operation. Each circle represents the value for an individual rat. Circles with bars indicate the group mean ± SEM. The asterisk indicates a significant difference (p < 0.05) between groups.
MAP

that the "completeness" of baroreceptor deafferentation is impossible to measure of cardiac output, it is impossible to arterial pressure following ABD. In the absence of ABD (r = 0.21, n = 8) in this small group of rats. The increased resting arterial pressure produced by the study. There was not a significant correlation, however, between the reduction in baroreflex gain and the increase in mean arterial pressure (≈ 15 mm Hg) is clear, based on the demonstration that baroreflex (pressure-heart function was markedly attenuated. This conclusion is supported further by the observation that ganglion-blockade normalizes arterial pressure in ABD rats. The hemodynamic mechanism by which autonomic blockade lowered arterial pressure in ABD rats, however, is not known. A reduction in vascular resistance by inhibition of sympathetic nerve vasoconstriction, or a reduction in cardiac output via uncompensated venodilation could conceivably be responsible. Direct measurements of cardiac output would be necessary to answer this question. Of course, these findings do not irrefutably establish the existence of increased sympathetic nerve traffic in rats with ABD, but the failure to demonstrate increased vascular reactivity to norepinephrine or sympathetic nerve stimulation (unpublished observations) in rats with ABD make it likely possibility. Since the elevated heart rates of rats with ABD were normalized (relative to sham-operated rats) by treatment with atropine, and not affected differently from that of sham-operated rats by treatment with propranolol, it can be concluded that sympathetic vasoconstrictor nerve traffic to the heart was the sole cause of the tachycardia in ABD rats. Thus, ABD produced a selective, rather than a diffuse, nonspecific increase in sympathetic neural activity.

In the present investigation, a mild, chronic hypertension was produced in rats by partial baroreceptor deafferentation. That overall baroreflex function was markedly attenuated is clear, based on the demonstration that baroreflex (pressure-heart period) gain and range were significantly reduced in conscious rats 1 month following ABD. The average increase in mean arterial pressure (≈ 15 mm Hg) is similar to that observed in most other recent studies of chronic neurogenic hypertension, although clearly in this study, some animals became hypertensive while others stayed in the normotensive range following ABD. The lack of a significant correlation between the reduction in baroreflex gain and the increase in arterial pressure produced by ABD suggests that the "completeness" of baroreceptor deafferentation alone could not explain these differences in arterial pressure following ABD. In the absence of measurements of cardiac output, it is impossible to determine whether arterial pressure was increased secondary to elevated cardiac output, total peripheral resistance, or both. A previous hemodynamic study of neurogenic hypertension in rats, however, and the failure here to normalize arterial pressure with total cardiac autonomic blockade (atropine plus propranolol), indicate a high likelihood that increased peripheral resistance was the predominant cause of hypertension in rats with ABD.

The normalization of arterial pressure by autonomic blockade, even 1 month following ABD, indicates that the hypertension was truly of "neurogenic" origin. This conclusion is supported further by the observation that ganglion-blockade normalized pressure in ABD rats. The hemodynamic mechanism by which autonomic blockade lowered arterial pressure in ABD rats, however, is not known. A reduction in vascular resistance by inhibition of sympathetic arteriolar vasoconstriction, or a reduction in cardiac output via uncompensated venodilation could conceivably be responsible. Direct measurements of cardiac output would be necessary to answer this question. Of course, these findings do not irrefutably establish the existence of increased sympathetic nerve traffic in rats with ABD, but the failure to demonstrate increased vascular reactivity to norepinephrine or sympathetic nerve stimulation (unpublished observations) in rats with ABD make it likely possibility. Since the elevated heart rates of rats with ABD were normalized (relative to sham-operated rats) by treatment with atropine, and not affected differently from that of sham-operated rats by treatment with propranolol, it can be concluded that reduced vagal activity to the heart was the sole cause of the tachycardia in ABD rats. Thus, ABD produced a selective, rather than a diffuse, nonspecific increase in sympathetic neural activity.

In the present study, arterial pressure variability was assessed by 1-hour recording sessions and 5-minute interval measurements. Although this method...
is less rigorous than others that have been utilized, it should be pointed out that the variability of pressure in normal rats found in this study agrees well with that found in similar rats over 1-hour periods using 40 Hz sampling times and computer-generated frequency histograms. Measurements in rats 1 month following ABD indicated a small but non-significant increase in arterial pressure variability relative to that of control rats. This finding is consonant with the reduced, but functional, baroreflex activity found in rats with ABD. A somewhat larger change in pressure variability might have been expected from rats with a nearly fourfold reduction in baroreflex gain, but two complicating factors confound such a straightforward extrapolation. First, the baroreflex curves were obtained at steady-state changes in pressure, while phasic reflex changes are more important in the beat-to-beat regulation that largely determines pressure lability. Second, the baroreflex curves obtained here related pressure to heart period, while the reflex control of vascular resistance undoubtedly contributes more to the determination of pressure variability. Of course, these factors also may partially explain the failure here to observe a correlation between the inhibition of baroreflex gain and the increase in arterial pressure following ABD. Nevertheless, since reduced baroreflex activity was sufficient to prevent the wide swings in pressure universally noted in totally barodenervated animals, arterial pressure measured casually in this study probably is a reasonable representation of actual time-averaged pressure levels in ABD rats. Consistent with this postulate of a more stable elevation in arterial pressure in ABD rats is the finding of a significantly increased heart weight to body weight ratio in rats 2 months following ABD (unpublished observations), in contrast to results from rats with total baroreceptor denervation where no such increase was observed despite a similar measured increase in arterial pressure. Few investigators have attempted to determine whether chronic total or partial baroreceptor denervation causes the hypertrophic cardiovascular alterations associated with most forms of chronic hypertension, although several early studies suggested the existence of cardiac hypertrophy in chronically "debuffered" animals.

Plasma volume in ABD rats was significantly lower than that of sham-operated control rats. This result is predicted by the systems model of Guyton et al. to occur in physiologic response to any increase in arterial pressure. Indeed, we have documented that fluid loss and fluid volume contraction occur immediately (within 24-48 hr) following ABD in rats. If it is assumed that volume contraction in this model is a result of hemodynamic alterations occurring with increased arterial pressure, the persistence of plasma volume contraction in ABD rats (up to 1 month) and the failure of volume contraction to persist in rats with total baroreceptor denervation offers further evidence that hypertension in ABD rats is a more stable variety than that seen following total baroreceptor denervation.

According to the systems model, volume contraction should lead eventually to a restoration of normal arterial pressure; this failed to occur in rats with ABD. Nevertheless, it is of considerable interest to note the positive correlation between plasma volume and mean arterial pressure in rats with ABD. It appears that those rats undergoing ABD that are least able to contract plasma volume during a neurogenically induced rise in arterial pressure maintain the highest level of arterial pressure in the chronic situation. Factors that could influence this ability to reduce vascular volume (presumably via increased renal fluid excretion) following ABD are increases in renal sympathetic nerve activity, ADH secretion, secretion of sodium-retaining hormones, or perhaps even intrinsic differences in renal excretory capacity. All of these factors would influence renal function in such a way as to require a higher arterial pressure to achieve a given renal fluid excretion: a rightward shift in the so-called "renal function curve" of Guyton et al. Whatever the explanation, it may not be possible to ascribe the existence of "neurogenic" hypertension in ABD rats exclusively to neurally mediated vasoconstriction. This observation perhaps could explain the apparent variability in the susceptibility of different individuals or species to the development of hypertension following total or partial baroreceptor denervation.

In conclusion, selective, aortic baroreceptor deafferentation (ABD) in the rat produces a partial reduction of overall baroreflex function and a chronic, mild hypertension. Variability of arterial pressure is increased only slightly in rats with ABD, consistent with the maintenance of intact (though attenuated) baroreflexes. The hypertension is apparently neurogenic, in that pressure can be normalized by total autonomic blockade. Plasma volume is reduced in rats with ABD. A significant correlation between plasma volume and arterial pressure in rats with chronic ABD, however, suggests that a relative elevation in vascular volume also may be a factor in the pathogenesis of this form of hypertension.

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