Salt-Sensitive Hypertension Caused by Long-term α-Adrenergic Blockade in the Rat


We conducted the present study to test the hypothesis that sympathetic responsiveness, rather than its absolute level of activity, is a determinant of salt-sensitive hypertension. Sprague-Dawley rats were instrumented for computerized recordings of arterial pressure and placed in metabolic cages. In one group (n=10), the α1-adrenergic antagonist prazosin was chronically infused throughout the experiment. A second group served as a vehicle control (n=9). Mean arterial pressure, sodium and water intake, urine output, and urinary sodium excretion were measured for 3 control days (0.4% NaCl diet), followed by 10 days of increased dietary NaCl (8.0% NaCl) and a subsequent 3-day recovery period (0.4% NaCl). Plasma renin activity was measured on day 2 of 0.4% NaCl, days 2 and 9 of 8.0% NaCl, and day 2 of the recovery period. Control values for all variables were similar between groups. Increased dietary NaCl resulted in a gradually developing hypertension in prazosin-treated rats. By day 10 of the 8% NaCl diet, arterial pressure had increased significantly more in prazosin-treated (41±6 mm Hg) compared with vehicle (8±4 mm Hg) rats. There were no differences between groups for daily or cumulative sodium or water balances throughout the study. During 0.4% NaCl, plasma renin activity was similar in prazosin (2.9±0.8 ng/mL per hour) and vehicle (4.1±0.7 ng/mL per hour) groups and was equally suppressed during 8.0% NaCl. These results are consistent with the hypothesis that impaired adrenergic responsiveness, caused by prazosin infusion, is a determinant of salt-sensitive hypertension in the rat. (Hypertension 1993;21:995–999)

KEY WORDS • sympathetic nervous system • receptors, adrenergic • sodium • prazosin • hypertension, sodium-dependent

Although studies indicate that certain forms of salt-dependent hypertension are neurogenically mediated, the relation between changes in salt intake and sympathetic activity, and how that relation is altered in salt-sensitive individuals, is not clear. One hypothesis is that, under conditions of low to normal salt intake, salt-sensitive hypertensive patients have chronically elevated sympathetic activity, specifically to the kidney. This would shift the renal function curve to a higher pressure, resulting in blood volume expansion and hypertension when salt intake was elevated. A second hypothesis is that the responsiveness of the sympathetic nervous system, rather than its basal level, is abnormal in salt-sensitive individuals. For example, some studies suggest that increased salt intake stimulates sympathetic outflow in salt-sensitive rats and humans. Alternatively, other studies suggest that salt-resistant subjects suppress sympathetic activity in response to increased NaCl intake, whereas salt-sensitive subjects have an attenuated sympathoinhibitory response. Regardless of the nature of the abnormal sympathetic response, the salt-sensitive subject has an inappropriately high level of sympathetic activity under conditions of increased salt intake.

Hence, it is not clear which type of sympathetic dysfunction causes salt-sensitive hypertension: high basal sympathetic activity, salt-induced sympathoexcitation, or an attenuated sympathoinhibitory response to increased salt intake. The present study was conducted to address this issue. We measured the response of arterial pressure to increasing dietary sodium chloride (NaCl) in vehicle control rats and rats with chronic α-adrenergic blockade. We predicted that, if increased activity of the sympathetic nervous system, either basal or salt-induced, was a prerequisite for salt-sensitive hypertension, rats with α-adrenergic blockade would be salt resistant. On the other hand, if inability to withdraw sympathetic tone to vascular and renal effectors was the primary cause of salt-dependent hypertension, rats with long-term α-adrenergic receptor blockade would be salt sensitive because the salt-induced withdrawal of sympathetic activity would be ineffective.

Methods

All procedures were conducted in accordance with institutional and National Institutes of Health guidelines. Male Sprague-Dawley rats (275–325 g, Harlan Sprague Dawley, Inc., Indianapolis, Ind.) were prepared for catheter implantation using aseptic technique. Arterial and venous catheters were advanced to the abdominal aorta and vena cava, respectively, from the femoral vessels. The distal ends of the catheters were tunneled subcutaneously to the head, where they were secured to the skull with stainless-steel screws and dental acrylic. The catheters were then passed through a lightweight flexible spring connected to a single-channel hydraulic swivel to which the venous catheter was attached. The incisions were closed, and a single
dose of penicillin G (100,000 units i.m.) was administered. On recovery from anesthesia, rats were placed in individual metabolic cages with the swivels mounted above. A 24-hour per day infusion of either sterile water (vehicle) or the α1-adrenergic receptor antagonist prazosin (3 mg/day; Pfizer, Groton, Conn.) dissolved in sterile water was started. Both vehicle and prazosin were infused through a 0.2-μm syringe filter at a volume flow rate of 7 mL/day. Rats were allowed 2–3 days to recover from surgery before the experimental protocol was begun. A 0.4% NaCl diet (Research Diets, New Brunswick, N.J.) and distilled water were provided ad libitum throughout the recovery period.

The protocol consisted of measuring the cardiovascular and fluid balance responses to increased dietary NaCl in the control rats (vehicle; n=9) and rats with impaired sympathetic responsiveness (prazosin; n=10). Three days after catheter implantation, daily measurements of mean arterial pressure, heart rate, food intake, water intake, and urine output were begun. All variables were measured in conscious, unrestrained rats in their home cage. Measurements were made for 16 consecutive days that were divided into three experimental periods: 3 control days (0.4% NaCl diet), 10 days of increased NaCl intake (8.0% NaCl, Research Diets), and a 3-day recovery period (0.4% NaCl diet).

Mean arterial pressure was measured by connecting the arterial catheter to a pressure transducer coupled to a polygraph (Grass Instrument Co., Inc., Quincy, Mass.). The arterial pressure signal was monitored for 30 minutes by computer at a sampling rate of 1 Hz as previously described. Heart rate was measured by increasing the chart speed and counting peaks on the pulsatile pressure tracing. Twenty-four-hour food and water intakes and urine output were measured gravimetrically. Sodium intake was calculated from food intake (grams per day) and sodium content of the diet, which had previously been determined by flame photometry (0.4% NaCl, 0.07 mEq/g; 8.0% NaCl, 1.00 mEq/g). Urinary sodium concentration was measured with an ion-specific electrode (Nova Biomedical, Waltham, Mass.). Urinary sodium excretion was calculated as the product of urine flow rate and urinary ion concentration.

In addition, plasma renin activity was measured on 4 separate days: the second control day, days 2 and 9 of 8.0% NaCl, and day 2 of the recovery period. On each of these days, a 500-μL blood sample was collected into a chilled 1-mL syringe containing 1 mg EDTA in a volume of 20μL. Sample blood was centrifuged and stored at −70°C for later radioimmunoassay as previously described.

Finally, to test the efficacy of α-adrenergic receptor blockade, pressor responses to intravenous bolus injections of the α-agonist phenylephrine (3.5 μg) were measured in both groups during the control (day 2), high NaCl (day 5), and recovery (day 2) periods.

Statistical Analysis

Values for the 3 control days were averaged to obtain a single control value for each group. Between-group comparisons of these control values were made with the unpaired Student's t test. For comparisons of the responses to increased dietary NaCl between vehicle and prazosin groups, data were first normalized by calculating the change from control for each group. Between-group comparisons were then made by analysis of variance. A significant F ratio was followed by Duncan's multiple-range test to test for differences between groups for specific days. A value of p<0.05 was considered statistically significant for all tests. Results are reported as mean±SEM.

Results

During the control period (0.4% NaCl), there was no statistical difference in mean arterial pressure between vehicle (107±2 mm Hg) and prazosin-treated (112±2 mm Hg) rats. However, long-term prazosin administration resulted in a marked, salt-induced hypertension (Figure 1). Compared with vehicle rats, mean arterial pressure was increased significantly more in prazosin-treated rats on day 1 of 8.0% NaCl and continued to increase over the next 9 days. By day 10, mean arterial pressure had risen 41±6 mm Hg in prazosin-treated rats compared with 8±4 mm Hg in vehicle rats. By the end of the 3-day recovery period (0.4% NaCl), mean arterial pressure returned to control levels in both groups.

Heart rate was significantly lower in vehicle (378±6
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FIGURE 2. Line plots show NaCl-induced changes in sodium (left panels) and water balance (right panels) variables in vehicle and prazosin-treated rats. Changes were calculated from the average of 3 control days (0.4% NaCl) before NaCl intake was increased. *Statistically significant difference between vehicle and prazosin groups.

 beats per minute) compared with prazosin-treated rats (422±11 beats per minute). Increasing dietary NaCl had negligible effects on heart rate in both groups (Figure 1). Pressor responses to phenylephrine injection were measured on the second control day, day 5 of high NaCl, and day 2 of the recovery period. Responses in vehicle rats were 40±3, 52±2, and 57±3 mm Hg, respectively, compared with −5±2, −5±2, and −2±1 mm Hg in prazosin-treated rats.

In regard to sodium and water balance, there were no differences between vehicle and prazosin-treated rats for sodium intake (0.8±0.1 versus 0.9±0.1 mEq/24 hours), urinary sodium excretion (0.8±0.1 versus 0.6±0.1 mEq/24 hours), water intake (30.4±4.0 versus 31.4±5.5 mL/24 hours), or urine output (25.6±3.7 versus 23.1±4.9 mL/24 hours) during the 0.4% NaCl diet. As expected, sodium intake and excretion increased when dietary NaCl was increased to 8.0%, reaching steady-state levels of 15–20 mEq/24 hours above control (Figure 2). Despite an equivalent increase in sodium intake, water intake increased twice as much in prazosin-treated as vehicle rats (Figure 2). Urine output followed a similar pattern. There were no differences in cumulative sodium or water balance between the two groups at any point in the study (Figure 3). Finally, plasma renin activity was similar in both groups during the control period and was equally suppressed when NaCl intake was elevated (Figure 4). This was followed by a partial recovery within 2 days of a return to a 0.4% NaCl diet.

Discussion

Although several studies suggest that some forms of salt-sensitive hypertension are caused by impaired sympathetic control of the circulation, the mechanisms are not well understood. Theoretically, neurogenic salt-dependent hypertension may be caused by three types of sympathetic dysfunction: 1) chronically elevated tonic renal sympathetic nerve activity, 2) salt-induced activation of the sympathetic nervous system, or 3) an attenuated sympathoinhibitory response to increased salt intake.

In the present study, long-term administration of an α1-adrenergic receptor antagonist resulted in a marked hypertensive response to increased salt intake. Based on this observation alone, it is clear that an intact sympathetic nervous system is required for arterial pressure to be maintained within normal limits when salt intake is elevated. More specifically, we interpret these results to be consistent with the latter hypothesis stated above; that is to say, sympathetic outflow is normally suppressed during periods of increased salt intake. This would result in responses of the vasculature (vasodilation) and kidney (natriuresis), which would maintain arterial pressure within normal limits despite large increases in NaCl intake. It would be expected that any
intervention that attenuates this NaCl-induced sympathoinhibition would result in salt-sensitive hypertension. Indeed, in the present study, salt-induced hypertension in prazosin-treated rats may be explained by the fact that neurally mediated vascular and renal responses to increased salt intake were blocked at the peripheral effector site. As a result, any decrease in sympathetic nerve discharge, either to the vasculature or kidney, was ineffective because the receptors were blocked.

The underlying assumption of the present study is that prazosin, a selective α1-adrenergic receptor antagonist, significantly attenuates sympathetic input to vascular smooth muscle and renal tubular cells. It is well established that increases in vascular smooth muscle tone and proximal tubule sodium and water reabsorption in response to sympathetic stimulation are mediated, in part, by α1-receptors. Although these responses may also be regulated by neurotransmitters that bind to other receptor types, there is no question that prazosin significantly reduces the response of these sites to both basal and elevated sympathetic nerve activity.

Based on this assumption, salt-sensitive hypertension in prazosin-treated rats cannot be explained by high basal sympathetic tone to the kidney. Although β-adrenergic receptor-mediated effects of renal sympathetic nerve activity on renin secretion were not blocked in this study, basal plasma renin activity was not elevated in prazosin rats. Moreover, plasma renin activity was suppressed to nearly undetectable levels when dietary NaCl was increased to 8.0%. These results are also inconsistent with the idea that the hypertension was due to salt-induced sympathoexcitation, because arterial pressure did not increase in the vehicle group but did in the group in which α-adrenergic receptors were blocked.

Although our results are compatible with a long-term suppression of sympathetic outflow in response to increased salt intake, definitive proof awaits the development of a reliable technique for measuring sympathetic activity over long periods of time (days to weeks) in awake animals and humans. Moreover, the mechanism of long-term salt-induced sympathoinhibition remains to be established. Recently, our laboratory and others have reported that denervation of arterial baroreceptors also results in salt-sensitive hypertension in the rat. This observation suggests that an intact arterial baroreceptor reflex is necessary to prevent salt-induced hypertension, presumably by decreasing sympathetic activity. This concept is further supported by reports of impaired arterial and cardiopulmonary baroreceptor reflexes in the Dahl salt-sensitive rat, a genetic model of salt-sensitive hypertension. Indeed, it has been shown that the Dahl salt-sensitive rat has an impaired ability to suppress sympathetic activity when dietary salt is increased. Clinical reports are consistent with this observation, in that salt-resistant subjects have lower plasma concentrations of norepinephrine and epinephrine when dietary salt is increased compared with salt-sensitive subjects.

Another interesting finding in this study was that salt-induced polydypsia and polyuria were exaggerated in prazosin-treated rats compared with vehicle controls. We have no explanation for this finding, but several possibilities exist. Because α-adrenergic receptor blockade inhibits sodium reabsorption in the proximal tubule, one may speculate that prazosin-treated rats would excrete more sodium, and therefore water, than vehicle-treated rats when dietary sodium was increased. However, this was not supported by measurements of
cumulative sodium balance. On the contrary, there was a tendency for vehicle rats to retain more sodium than prazosin-treated rats, although there was no statistical difference between the two groups. Another possible explanation is that prazosin has central effects on either vasopressin release or drinking behavior. Indeed, intravenously administered prazosin acts centrally to suppress sympathetic activity at doses lower than those used in this study.20 The possibility that prazosin acts centrally to alter drinking behavior, vasopressin release, or both remains to be investigated.

References

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