Does Renin Determine the Blood Pressure Response to Calcium Entry Blockers?

BERNARD WAEBER, JÜRGEN NUSSSBERGER, AND HANS R. BRUNNER

SUMMARY Male Wistar rats with one-kidney, one clip renal hypertension were maintained on either a regular or a low salt diet for 3 weeks after clipping. At that time mean blood pressure in the unanesthetized rats was equally elevated in sodium-depleted \((n=17)\) and in sodium-replete rats \((n=19)\), but plasma renin activity was significantly higher in the former \((p<0.05)\). Infusion of the calcium entry blocker verapamil at a rate of 0.05 mg/kg/minute decreased blood pressure within 60 minutes to a similar extent in rats kept on a salt-deficient diet and in rats fed a regular salt diet. In all rats taken as a group, there was a close, direct correlation \((r=0.87, p<0.001)\) between the magnitude of the blood pressure response to verapamil and the pretreatment blood pressure levels. Verapamil markedly accelerated heart rate and stimulated renin release in all rats. In additional groups of sodium-depleted \((n=8)\) and sodium-replete renal hypertensive rats \((n=7)\), nifedipine administration \((4 \mu\text{g/kg/min i.v.})\) within a 45-minute observation period caused a blood pressure fall \((p<0.001)\) and heart rate acceleration \((p<0.001)\) that were comparable in both groups. These findings suggest that in the rat with renal hypertension the short-term blood pressure response to the calcium antagonists verapamil and nifedipine is not influenced by the state of sodium balance and plasma renin activity. In this experimental model of hypertension, the magnitude of the blood pressure lowering effect of calcium entry blockers appears to be proportional to pretreatment blood pressure levels. (Hypertension 7:223-227, 1985)

KEY WORDS • renal hypertension • verapamil • nifedipine • conscious rats • high and low renin levels

During recent years numerous studies have focused on the crucial role of intracellular free calcium in determining vascular smooth muscle tone. \(^1\) \(^2\) Calcium channel blocking agents have been used to reduce intracellular calcium in an attempt to lower the blood pressure of hypertensive patients. Such drugs have proved to be helpful in managing hypertensive patients and appear particularly effective in reducing blood pressure when the rate of renin secretion is low. \(^3\) \(^4\)

The present investigation was undertaken in conscious hypertensive rats to assess the effect of a change in sodium balance and renin release on the short-term blood pressure response to the calcium entry blockers verapamil and nifedipine. \(^5\) The experiments were carried out in rats with hypertension induced by partially occluding one renal artery with the contralateral kidney removed (one-kidney, one clip renal hypertension) maintained postoperatively for 3 weeks on either a salt-deficient or a regular salt intake. As was known from previous work, \(^6\) \(^7\) blood pressure during the phase of established hypertension achieves similar high levels in sodium-depleted and sodium-replete rats in spite of the fact that plasma renin activity is elevated only in the former.

Materials and Methods

Male Wistar rats weighing 140 to 180 g (Madörin AG, Füllinsdorf, Switzerland) were used for this study. They were housed in a room with a constant temperature of 23°C and a humidity of about 50%. A solid silver clip (0.2 mm inside diameter) was placed on the left renal artery, and a right nephrectomy was performed with the rats under ether anesthesia. The animals were then returned to their cages and given a regular rat chow diet (Indulab, Buchs, Switzerland) containing 0.113 mmol/g of sodium. Two days later, this diet was replaced in half of the rats by a salt-deficient diet (Indulab, Buchs, Switzerland) contain-
TABLE 1. Basal Characteristics and Plasma Renin Activity of the Study Groups

<table>
<thead>
<tr>
<th></th>
<th>Sodium-replete rats</th>
<th>Sodium-depleted rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle (n = 9)</td>
<td>Verapamil (n = 10)</td>
</tr>
<tr>
<td></td>
<td>Vehicle (n = 9)</td>
<td>Verapamil (n = 8)</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>233 ± 12</td>
<td>214 ± 43</td>
</tr>
<tr>
<td>Mean blood pressure (mm Hg)</td>
<td>195 ± 5</td>
<td>204 ± 9.2</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>407 ± 15</td>
<td>425 ± 14</td>
</tr>
<tr>
<td>Plasma renin activity (ng/ml/hr)</td>
<td>4 ± 1</td>
<td>56 ± 134</td>
</tr>
</tbody>
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*p < 0.05, †p < 0.01, sodium-depleted versus sodium-replete rats.
 tp < 0.01, ††p < 0.001, verapamil versus vehicle.
All values are means ± SEM.

ing only 0.002 mmol/g of sodium. Food and tap water were provided ad libitum throughout the study.

Three weeks postoperatively, under light ether anesthesia, all animals had the right external iliac artery cannulated with a PE-50 catheter and the right femoral vein with a PE-10 catheter. Both tubings were filled with a heparinized 5% dextrose solution. The rats were then placed in a plastic tube to restrict their movements, where they were allowed to wake up. Arterial pressure and heart rate were monitored with a transducer (Statham, Hato Rey, Puerto Rico) connected to an electrogalvanometer (Philips 2000, Eindhoven, Netherlands) and recorded on a light-sensitive oscillograph (Mannarp 150, Electronic Institute Limited, London, England). The experiment was started 90 minutes after the end of the anesthesia, when the rats' blood pressure and heart rate had stabilized.

In the first set of experiments four groups of rats were studied. In one group of sodium-replete rats as well as in one group of sodium-depleted animals, blood pressure and heart rate were recorded during a 60-minute infusion of verapamil. Control experiments were carried out in sodium-replete and sodium-depleted rats infused with vehicle. In all these rats, after a 60-minute infusion of either verapamil or its vehicle, a blood sample (2 ml) was drawn through the arterial line for determination of plasma renin activity.

Verapamil (Isoptin, 5 mg/2 ml) was dissolved in 5% dextrose to achieve a concentration of 1.25 mg/ml and was administered at a dose of 0.05 mg/kg/minute. Either the active drug or its vehicle was infused at a constant rate (40 /μL/kg/min) with a syringe pump (model 455, Sage Instrument Co., White Plains, NY). Plasma renin activity was determined by radioimmunoassay.8

In a second set of experiments, to sodium-replete and sodium-depleted rats prepared as described previously, nifedipine (Adalat, 0.2 mg/2 ml) was administered instead of verapamil. Nifedipine was infused intravenously for 45 minutes at a dose of 4 μg/kg/minute (40 μL/kg/min). Throughout the observation period the catheter and the syringe containing the nifedipine solution were wrapped in aluminum foil to prevent light-induced deterioration of the compound.

Data are reported as means ± 1 standard error of the mean (mean ± SEM). Statistical evaluation of the results was made with one-way analysis of variance and, where appropriate, the Student's t test for unpaired data. Correlation coefficients were calculated with the rank correlation test. The probability level of less than 0.05 was considered significant.

Results

During the 3 weeks after renal artery clipping, the weight gain in sodium-replete (n = 19) and sodium-depleted (n = 17) rats subsequently given verapamil or its vehicle averaged 59.5 ± 5.6 and 47.7 ± 3.5 g respectively (p > 0.05, sodium-replete versus sodium-depleted rats). The basal characteristics of the study groups before administration of verapamil or its vehicle are outlined in Table 1. On the day of the experiment, no significant difference in body weight, base-
Figure 2. Effect of vehicle administration on blood pressure and heart rate of sodium-replete and sodium-depleted conscious rats with one-kidney, one clip renal hypertension.

Figure 3. Relationship between baseline mean blood pressure and mean blood pressure response to administration of verapamil in sodium-replete and sodium-depleted conscious rats with one-kidney, one clip renal hypertension.

A close correlation ($r = 0.87, n = 18, p < 0.001$) appeared between these two parameters.

Plasma renin activity was measured in all rats at the end of the experiment (Table 1). As expected, renin secretion was markedly higher in sodium-depleted than in sodium-replete rats, in both the vehicle- and verapamil-treated rats. Verapamil infusion markedly increased plasma renin activity in sodium-replete as well as in sodium-depleted rats.

Table 2 summarizes the results obtained with nifedipine in sodium-replete ($n = 7$) and sodium-depleted ($n = 8$) renal hypertensive rats. On the day of the experiment there was no significant difference in body weight between the former (233 ± 9 g) and the latter (214 ± 7 g). The blood pressure decrease and the heart rate acceleration induced by this compound were not dependent on the salt intake. Within 45 minutes, blood pressure fell by 43 ± 9.9 mm Hg in sodium-replete rats and by 45 ± 6 mm Hg in sodium-depleted rats. In all animals taken as a group there was a significant correlation between blood pressure before nifedipine

<table>
<thead>
<tr>
<th>TABLE 2. Blood Pressure and Heart Rate Effect of Nifedipine Administration</th>
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<tr>
<td>Before nifedipine</td>
</tr>
<tr>
<td>Mean blood pressure (mm Hg)</td>
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<tr>
<td>Sodium-replete rats ($n = 7$)</td>
</tr>
<tr>
<td>Sodium-depleted rats ($n = 8$)</td>
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<tr>
<td>Heart rate (beats/min)</td>
</tr>
<tr>
<td>Sodium-replete rats</td>
</tr>
<tr>
<td>Sodium-depleted rats</td>
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</tbody>
</table>

*p < 0.05, †p < 0.001, after versus before nifedipine administration. All values are means ± SEM.
administration and the magnitude of the drug-induced reduction observed at the end of the experiment (r = 0.60, p < 0.05).

Discussion

Free intracellular calcium has been demonstrated to play a key role in regulating the contractile state of the vascular smooth muscle cell. Evidence emerging during the last few years has suggested that the development of both genetic hypertension in the rat and essential hypertension in humans is linked to an abnormality in cellular calcium metabolism. It now appears possible that the cellular defect of calcium handling is caused primarily by a derangement in transmembranous sodium transport. In support of such a mechanism is the recent observation that calcium channel blockers, which inhibit the flux of extracellular calcium ions across the cell membrane, have the greatest antihypertensive effect in patients with low plasma renin activity. One can wonder whether the discrepancy between the experimental and clinical results is related to the genetic nature of essential hypertension, which may be expressed, for instance, by an abnormal transmembranous ionic transport. Of course, other factors such as age and severity of the hypertensive disease could have influenced the antihypertensive response of patients to calcium antagonists.

In this study two different calcium channel blockers, verapamil and nifedipine, were administered to rats with the same hypertension model because verapamil, unlike nifedipine, has been shown to interact in vitro with α-adrenergic receptors. Such an action unrelated to calcium channel blockade could potentially interfere with the interpretation of our results. The fact that we obtained comparable results with both inhibitors suggests that α-blockade was not the main mechanism responsible for the blood pressure lowering effect of verapamil.

On the other hand, in several aspects the present experimental findings confirm those previously observed in hypertensive patients during short-term blockage of calcium channels. First, as in the patients, heart rate was accelerated during verapamil and nifedipine infusion in both sodium-depleted and sodium-replete rats, most probably as a consequence of a baroreceptor reflex-mediated increase in sympathetic nerve activity. Second, as in hypertensive patients renin secretion was markedly stimulated by verapamil administration in rats. The reduction in arterial pressure per se as well as the compensatory stimulation of sympathetic nerve activity may well be responsible for the effect of verapamil on renin release. Finally, in all rats taken as a group, the magnitude of the blood pressure decrease induced by both verapamil and nifedipine administration was directly proportional to the pretreatment blood pressure level. Again, this finding is in total agreement with clinical experience.

Conclusion

Our data indicate that the acute vasodilating effect of calcium entry blockade in the conscious rat with renal hypertension does not depend on the state of sodium balance nor on the degree of activation of the renin-angiotensin system, but that it is directly related to pretreatment blood pressure.

Acknowledgment

We thank Mr. A. Lincoln for expert technical assistance and Ms. A.-F. Stalé and A. Campiche for typing the manuscript.

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Hypertension. 1985;7:223-227
doi: 10.1161/01.HYP.7.2.223

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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