Development of Hypertension in Animals With Reduced Total Peripheral Resistance

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The object of the present study was to determine whether deoxycorticosterone acetate (DOCA)-salt hypertension can be produced in rats in the presence of low total peripheral resistance (TPR) induced by long-term administration of minoxidil, a vasodilator. The rats were divided into four groups: sham-control, DOCA-salt, minoxidil, and DOCA-salt with minoxidil. The rats in both DOCA groups had DOCA pellets implanted subcutaneously and were given saline to drink. The rats in both minoxidil groups were given minoxidil (3 mg/day) in the drinking water throughout the experiment. Final measurements, including mean arterial blood pressure, cardiac index, and renal blood flow were made after 4–6 weeks. Flesseves in the body were made using radioactive microspheres. Cardiac index (ml • min⁻¹ • 100 g⁻¹) in sham-control rats averaged 18±2 and was higher in the other groups: 23±4 (DOCA-salt), 25±2 (minoxidil), and 30±2 (DOCA-salt plus minoxidil). Mean arterial pressure (mm Hg) was increased in both DOCA-salt rats (160±8) and DOCA-salt plus minoxidil rats (153±5) as compared with sham-control (116±2) and minoxidil (113±2) rats. There was no significant difference in TPR between the sham-control and DOCA-salt rats, but TPR in minoxidil and DOCA-salt plus minoxidil rats was 30% and 28% lower than that in untreated sham-control and DOCA-salt hypertensive rats, respectively. In contrast, renal vascular resistance was significantly increased in both DOCA-salt groups as compared with non-DOCA-salt groups. The results of this study show that long-term administration of minoxidil does not prevent the development of DOCA-salt hypertension, suggesting that an increase in TPR is not a necessary step in the development of DOCA-salt hypertension. Instead, increases in cardiac output were associated with the hypertension in all three experimental groups. These increases in cardiac output were more than sufficient to compensate for the decreases in TPR in the minoxidil and DOCA-salt plus minoxidil groups. (Hypertension 1992;20:828–833)

KEY WORDS • deoxycorticosterone • hypertension, mineralocorticoid • vascular resistance • minoxidil • microspheres

Considerable evidence has been accumulated to demonstrate that renal function, not total peripheral resistance (TPR), plays the central role in the long-term control of arterial blood pressure and the pathogenesis of hypertension. Consequently, an increase in TPR would be expected to increase arterial blood pressure only transiently if there is not a concomitant increase in renal vascular resistance. This hypothesis can be explained by the infinite gain principle of the renal–body fluid feedback mechanism as proposed in earlier studies from this laboratory. This principle states that any increase in arterial blood pressure will initiate pressure diuresis and natriuresis, which will continue to operate until blood pressure has returned to the normal level.

The basic hypothesis is that changes in TPR in all of the blood vessels in the body other than the kidney will not affect long-term control of arterial blood pressure. This hypothesis is supported by the finding that in clinical conditions such as beri beri, arteriovenous fistula, anemia, hyperthyroidism, pulmonary disease, Paget’s disease, removal of four limbs, and hypothyroidism, TPR is chronically increased or decreased, but blood pressure is normal. Instead of a change in blood pressure, there are reciprocal changes in cardiac output.

In animal experiments, we have previously demonstrated that a reduction in TPR due to the opening of an arteriovenous fistula does not change the long-term level of arterial blood pressure in both normal and deoxycorticosterone acetate (DOCA)-salt hypertensive rats. DOCA-salt hypertension can still develop in the presence of an arteriovenous fistula, although the rise in arterial blood pressure was less than in DOCA-salt hypertensive rats without fistula. Our fistula experiments have, to some extent, suggested that an increased TPR is not a necessary step in the development of DOCA-salt hypertension. However, it could be argued that the opening of an arteriovenous fistula did not decrease the resistance in the systemic circulation. The systemic resistance was actually increased in DOCA-salt hypertensive rats with or without fistula.

This increase of total systemic resistance may compensate for the decrease in TPR induced by the opening of an arteriovenous fistula. Whether DOCA-salt hypertension can be produced in animals with reduced total
systemic resistance by long-term administration of a peripheral vasodilator has not been previously studied.

Therefore, the main objective of the present study was to determine whether a reduction in TPR induced by the long-term administration of minoxidil can prevent the development of DOCA-salt hypertension. This would further test the hypothesis that a decrease in TPR without a concomitant decrease in renal vascular resistance will not lower blood pressure in hypertensive animals.

Methods

Animal Preparations

Approval to conduct this study was granted by our Institutional Animal Care and Use Committee in accordance with guidelines set forth by the National Institutes of Health.

All experiments were conducted in male Sprague-Dawley rats (University of Mississippi Medical Center, Jackson, Miss.) weighing 175–225 g. The 40 rats were divided into four groups (eight of the 40 rats [one in the minoxidil group, three in the DOCA-salt group, and four in the DOCA-salt plus minoxidil group] died for unknown reasons during the experiments and were excluded from the data analysis): sham-control (n=10), minoxidil (n=9), DOCA-salt (n=7), and DOCA-salt plus minoxidil (n=6). At the beginning of the experiment, the rats were anesthetized with pentobarbital sodium (50 mg/kg), and a unilateral nephrectomy was performed through a midline abdominal incision. The rats in both the DOCA-salt and the DOCA-salt plus minoxidil groups received DOCA pellets (75 mg, Innovative Research of America, Toledo, Ohio) subcutaneously. The rats in both the minoxidil and the DOCA-salt plus minoxidil groups were given minoxidil (Sigma Chemical Co., St. Louis, Mo.) at a dose of 3 mg/day in the drinking water throughout the 4–6-week experimental period. The rats in the sham-control group were maintained on standard rat diet and tap water ad libitum. The rats in the DOCA-salt and DOCA-salt plus minoxidil groups were given 0.9% saline instead of tap water for drinking. The rats in the minoxidil group were given tap water.

At the end of 4–6 weeks, the animals were anesthetized with sodium pentobarbital. The carotid artery was cannulated with polyethylene tubing (No. 7411, 0.5 mm i.d., 0.97 mm o.d., Clay-Adams, Parsippany, NJ.) for measurement of mean arterial blood pressure (MAP).

The jugular vein was cannulated with similar polyethylene tubing, and the catheter was advanced into the right atrium for the measurement of right atrial pressure. The location of the right atrial catheter was verified at the end of the experiment to ensure that the catheter was located in the right atrium. The femoral artery was cannulated with polyethylene tubing for the withdrawal of a reference blood sample for the determination of cardiac output and tissue flows. The catheters were prefilled with heparinized saline (1,000 units/ml). MAP was measured with a pressure transducer (P23ID, Statham Division, Gould Inc., Oxnard, Calif.) coupled to a polygraph (model 7D, Grass Instruments Co., Quincy, Mass.). Right atrial pressure was measured using a Statham venous pressure transducer (P23V). Both MAP and right atrial pressure were recorded for 30 minutes, and the values reported in the present study were the average pressure for 30 minutes. The heart rate (beats per minute) was determined from the pulse pressure recording.

Determination of Cardiac Output and Organ Flows

After the pressure measurements were taken, cardiac output and all other tissue flows were determined by using a microsphere technique reported previously. Briefly, the carotid catheter was advanced into the left ventricle for microsphere injection. Radioactive microspheres (15±3 μm, New England Nuclear, Boston, Mass.) labeled with Scandium-46 were injected into the left ventricle in each of the rats. A 1-ml reference blood sample was withdrawn from the left femoral artery, and the sampling time was recorded.

At the end of the experiment, the brain, heart, lungs, liver, spleen, stomach, intestine, testis, and skeletal muscle were removed, and individual organ flows were determined.

Analytical and Statistical Method

The blood samples for the measurements of hematocrit, plasma renin activity, and atrial natriuretic peptide (ANP) were taken after the cardiac index (CI) measurement. Hematocrit was determined by the microcapillary method. Plasma renin activity was measured in plasma buffered to pH 7.4 by a modification of the method described by Haber et al. Plasma ANP concentration was measured by radioimmunoassay as previously described. All plasma samples were run in a single assay whenever possible. Experimental data were compared using a 2 by 2 factorial analysis of variance. All data are reported as mean±SEM. Comparisons were made between DOCA-salt and non-DOCA-salt groups and between minoxidil- and non-minoxidil-treated groups. A value of p<0.05 was accepted as a statistically significant difference.

Results

Figure 1A shows the MAP responses in both normal and DOCA-salt hypertensive rats with and without minoxidil treatments. As expected, the rats in the DOCA-salt group showed a significant increase in MAP from 116±2 to 160±8 mm Hg (p<0.01). In addition, the rats in the DOCA-salt plus minoxidil group also showed a significant increase in MAP from a value of 113±3 for minoxidil treatment alone to a value of 153±5 mm Hg for DOCA-salt plus minoxidil treatment (p<0.01). Minoxidil treatment alone failed to significantly change MAP compared with the non-minoxidil-treated animals (p>0.05).

The data for CI (cardiac output per 100 grams body weight) are shown in Figure 1B. Both DOCA treatment and minoxidil treatment resulted in significant increases in CI (p<0.05 and p<0.01, respectively). The average CI of rats in the sham-control group was 18±2 ml · min⁻¹ · 100 g⁻¹. The mean CI of rats in the DOCA-salt group was 23±4 ml · min⁻¹ · 100 g⁻¹. The minoxidil-treated rats in both minoxidil and DOCA-salt plus minoxidil groups had average values for CI of 25±2 and 30±2 ml · min⁻¹ · 100 g⁻¹, respectively. These values of CI for the minoxidil-treated animals represent a 39% and 67% increase over the sham-control values. In

Huang et al  Hypertension and Hemodynamics 829
FIGURE 1. Bar graphs show changes in mean arterial blood pressure (panel A), cardiac index (panel B), and total peripheral resistance (panel C) in both normal and deoxycorticosterone acetate (DOCA)-salt hypertensive rats with and without long-term minoxidil treatment. SCTR, sham-control; MX, minoxidil. Values are mean±SEM. + and ** represent significance (p<0.05 and p<0.01, respectively) due to DOCA-salt treatment.

In addition, the value of CI in DOCA-salt plus minoxidil rats was 30% higher than the DOCA-salt only rats.

The data for calculated TPR, computed as MAP/Ci, are shown in Figure 1C. DOCA treatment did not produce a significant change in TPR (p>0.05). TPR of rats in the DOCA-salt group averaged 7.8±0.8 mm Hg · ml⁻¹ · min⁻¹ · 100 g⁻¹, a value that was not significantly different from the sham-control value of 7.0±0.8 mm Hg · ml⁻¹ · min⁻¹ · 100 g⁻¹. In contrast, minoxidil treatment resulted in significant decreases in TPR in both groups of animals (p<0.01). The rats in the minoxidil group showed a significant decrease in TPR with an average value of 4.9±0.6 mm Hg · ml⁻¹ · min⁻¹ · 100 g⁻¹. This value represents a decrease of 30% from the sham-control value. TPR in the DOCA-salt plus minoxidil rats averaged 5.3±0.5 mm Hg · ml⁻¹ · min⁻¹ · 100 g⁻¹, a value that was 28% lower than the DOCA-salt only animals.

The changes in renal blood flow and renal arteriolar resistance in both normal and DOCA-salt hypertensive rats are shown in Figure 2. The sham-control values for renal blood flow and renal vascular resistance averaged 5.0±0.6 ml · min⁻¹ · g⁻¹ and 27.2±4.2 mm Hg · ml⁻¹ · min⁻¹ · g⁻¹, respectively. The renal blood flow and renal vascular resistance for the rats in the minoxidil group were 5.2±0.6 ml · min⁻¹ · g⁻¹ and 28.8±7.7 mm Hg · ml⁻¹ · min⁻¹ · g⁻¹, values that are almost identical to the values observed in sham-control rats. The results of the present study would suggest that minoxidil treatment did not affect renal hemodynamics in normotensive rats. In contrast, the treatment with DOCA-salt alone resulted in a significant decrease in renal blood flow and an increase in renal vascular resistance (p<0.01). Mean values for renal blood flow and renal vascular resistance in the DOCA-salt group were 2.0±0.5 ml · min⁻¹ · g⁻¹ and 120.7±38.3 mm Hg · ml⁻¹ · min⁻¹ · g⁻¹. In addition, minoxidil-treated DOCA-salt rats also had a significant decrease in renal blood flow (p<0.01) and an increase in renal vascular resistance (p<0.01) as compared with control values observed in non-DOCA-salt-treated rats.
The average values of renal blood flow and renal vascular resistance in DOCA-salt plus minoxidil animals were 2.5±0.3 ml · min⁻¹ · g⁻¹ and 66.8±10.7 mm Hg · ml⁻¹ · min⁻¹ · g⁻¹, respectively.

The values for plasma renin activity, ANP, hematocrit, and right atrial pressure in response to the minoxidil treatment in both normal and DOCA-salt hypertensive rats are summarized in Table 1. There were no significant differences in plasma renin activity between minoxidil- and non-minoxidil-treated rats. But plasma renin activity in both the DOCA-salt and DOCA-salt plus minoxidil animals was significantly decreased compared with the non-DOCA-salt rats (p<0.01). Both DOCA-salt and minoxidil treatment resulted in significant increases in plasma ANP levels. DOCA-salt treatment resulted in a 60% increase in plasma ANP levels in the non-minoxidil-treated animals and a 110% increase in ANP in the minoxidil-treated animals. Minoxidil treatment resulted in a 90% increase in ANP in the non-DOCA-salt animals and a 147% increase in ANP in the DOCA-salt-minoxidil-treated animals. DOCA-salt treatment resulted in a significant decrease in hematocrit (p<0.01), whereas minoxidil treatment had no effect on hematocrit. Neither DOCA-salt nor minoxidil treatment had a significant effect on right atrial pressure (p<0.05).

The values for heart rate, heart weight, kidney weight, and body weight in both normal and DOCA-salt hypertensive rats during minoxidil treatment are summarized in Table 2. There were no significant changes in heart rate in any of these groups (p>0.05). The mean heart weight of rats (adjusted according to the body weight) in the sham-control group averaged 0.30±0.01 g/100 g. DOCA-salt treatment resulted in a significant increase in heart weight (p<0.01), whereas minoxidil treatment had no significant effect on heart weight (p>0.05). Heart weight of the rats in the minoxidil, DOCA-salt, and DOCA-salt plus minoxidil groups was 0.33±0.01, 0.42±0.02, and 0.44±0.02 g/100 g, respectively. The rats in the minoxidil group had a kidney weight identical (0.55±0.03 g/100 g) to the control rats. DOCA-salt treatment essentially doubled kidney weight (p<0.01) compared with control values in non-DOCA-salt rats; the rats in the DOCA-salt and DOCA-salt plus minoxidil groups had almost identical kidney weights with an average value of 1.1±0.1 g/100 g. Minoxidil treatment failed to show a significant effect on kidney weight. The rats in the DOCA-salt and DOCA-salt-plus-minoxidil-treated groups had a significant decrease in body weight (p<0.01). However, minoxidil treatment caused no significant changes in body weight (p>0.05). The decrease in body weight in the DOCA-salt hypertensive rats may be the sign of the onset of malignant DOCA-salt hypertension.12

Although the usual method for reporting blood flows measured by radioactive microspheres is in milliliters per minute per gram of tissue, it is also important in the present study to express kidney blood flow in terms of body mass. The reason is that renal control of arterial blood pressure often is related mainly to the ratio of kidney function to body mass. Renal blood flow expressed in this way (milliliters per minute per 100 grams body weight) were: control, 2.7±0.3; minoxidil, 2.8±0.3; DOCA-salt, 2.0±0.4; and DOCA-salt-plus minoxidil, 2.9±0.3. These numbers show that the renal blood flows per unit mass of the rats did not change significantly (p>0.05).

Table 1 and Figure 2 summarize the individual organ (or tissue) flows during minoxidil treatment in both the normal and DOCA-salt hypertensive rats. DOCA-salt treatment had no effect on organ (or tissue) flows in any of the organs (or tissues) measured (p>0.05). However, the blood flows to the spleen, testicles, stomach, large intesti-
The findings of the present study indicate that long-term minoxidil treatment (3 mg/day) for a period of 6 weeks did not significantly decrease the long-term MAP in normotensive rats. Minoxidil treatment only slightly decreased MAP in treated DOCA-salt hypertensive rats compared with the untreated DOCA-salt hypertensive rats (Figure 1A). DOCA-salt hypertension still developed during the minoxidil treatment, although TPR was not significantly decreased in both minoxidil-treated normotensive and DOCA-salt hypertensive rats, as compared with the untreated DOCA-salt hypertensive rats, respectively (Figure 1C). The observations of the present study are consistent with the data from Leenen and Prowse,13 who demonstrated that minoxidil lowers arterial blood pressure transiently in two-kidney, one-clip hypertensive rats for 1 week with a return of arterial blood pressure back to its original hypertensive level after 2-3 weeks of minoxidil treatment.

Based on the concept of the renal–body fluid feedback mechanism for arterial pressure control,1-4 the hypotensive effectiveness of minoxidil is highly dependent on changing renal vascular resistance and renal function. If minoxidil affected only peripheral resistance without affecting renal resistance it is predicted that minoxidil will only transiently decrease MAP because the renal body fluid mechanism will bring the arterial blood pressure back to its original hypertensive level. In the present study, minoxidil did not significantly affect renal blood flow and renal vascular resistance in normotensive rats. Minoxidil treatment slightly improves renal hemodynamics in DOCA-salt hypertensive rats, as seen by a 25% increase in renal blood flow and 45% decrease in renal arteriolar resistance (Figure 2). The renal observations in the present study are consistent with the reports of Gilmore et al14 and Zins,15 who showed there were no changes in renal blood flow and renal vascular resistance during minoxidil treatment. In addition, the study of Mitchell et al16 indicated that long-term treatment with minoxidil does not improve renal function in a majority of patients with benign hypertension, although it can markedly improve renal function in patients with malignant hypertension.

One of the interesting results in the present study is the rise in renal vascular resistance in both DOCA-salt–treated groups (Figure 2). Although the precise portion of the renal vessels responsible for the increase in renal vascular resistance is still the subject of controversy, evidence from the micropuncture studies of Dworkin et al17 have shown that afferent arteriole vascular resistance is decreased in DOCA-hypertensive rats. In a more recent study, Tojo et al18 have measured renal arteriolar diameter by using a vascular cast technique and have found that the afferent arterioles were constricted, whereas the efferent arterioles were dilated in DOCA-salt hypertensive rats. This pattern of the resistance changes in DOCA-salt hypertension may be a regulatory mechanism to protect the glomeruli from hypertensive damage.18 The precise mechanisms responsible for the increase in renal vascular resistance during DOCA-salt treatment is not yet known. A morphological study from Lee et al19 indicated that DOCA-salt hypertensive rats are frequently associated with significant structure alterations in the intimal, medial, and adventitial layers of vessel wall. Some of these changes mainly resulted from DOCA-salt treatment, but some of the changes appear to be secondary to the development of DOCA-salt hypertension.

Computer simulations conducted by Guyton and his colleagues2,2,20 demonstrated that dilating the arterioles and veins without dilating renal vasculature could not chronically decrease mean blood pressure. The differential alterations in TPR and renal vascular resistance in DOCA-salt hypertensive rats during long-term minoxidil treatment provided unique experimental evidence to support the computer simulation that a decrease in TPR without affecting renal vascular resistance does not lower high blood pressure. These results are compatible with our hypothesis that renal function, but not TPR, plays a predominant role in the long-term regulation of arterial blood pressure.
The maintenance of normal blood pressure in normotensive rats and high blood pressure in DOCA-salt hypertensive rats during long-term minoxidil treatment requires a substantial increase in cardiac output. As shown in Figure 1B, both normal and DOCA-salt hypertensive rats treated with minoxidil had CI increases of 39% and 67%, respectively, over the sham-control value. In addition, CI in minoxidil-treated hypertensive rats was 30% higher than in untreated DOCA-salt hypertensive rats. Thus, the extra increase in cardiac output offset almost all of the vasodilator effects of minoxidil.

The mechanism responsible for the minimal blood pressure decrease and the increase in cardiac output is mainly related to renal sodium and water retention. Several studies have suggested that minoxidil treatment results in increases in both plasma volume and blood volume. In addition, the decreases in hematocrit and increases in right atrial pressure, ANP (Table 1), and heart hypertrophy (Table 2) observed in the present study provide indirect evidence that hypercirculatory status does occur during long-term minoxidil treatment.

In addition to the overall hemodynamic changes, the distribution of the increased cardiac output to the individual tissues in response to the long-term administration of minoxidil was also studied. As seen in Table 3, minoxidil significantly increases blood flow to the spleen, stomach, large intestine, lungs, and testicles in both normal and DOCA-salt hypertensive rats. Blood flow to the heart in both normal and DOCA-salt hypertensive rats during minoxidil treatment was 20–25% higher than control but not significantly different because of a large variability. On the other hand, blood flow to the brain, liver, and muscles was generally unchanged. The general profile of organ (or tissue) blood flows observed in the present study is comparable with the reports by Humphrey and Zins.

In conclusion, long-term minoxidil treatment did not significantly change the long-term MAP in both normal and DOCA-salt hypertensive rats in the present study. DOCA-salt hypertension still developed in the face of a decrease in TPR caused by long-term minoxidil treatment. But this decrease in TPR only occurred in the peripheral circulation, with no decrease in renal vascular resistance, and in fact renal vascular resistance was increased in DOCA-salt–treated animals compared with non–DOCA-salt animals. Although there is a positive correlation between blood pressure and renal vascular resistance, the lack of a correlation between blood pressure and TPR suggests that renal function, but not TPR, plays a central role in the long-term regulation of blood pressure and the development of DOCA-salt hypertension. The implication of the present study is that a decrease in renal vascular resistance and improvement of renal function appear to be fundamentally essential for the treatment of hypertension. Any agent that can decrease TPR in the peripheral circulation only, without simultaneously decreasing renal vascular resistance, would not be expected to chronically lower blood pressure in hypertensive animals.

Acknowledgment

We thank Patty Sharp for performing the plasma renin activity and ANP assays.

References

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Hypertension. 1992;20:828-833
doi: 10.1161/01.HYP.20.6.828

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1992 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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