Renal Denervation Prevents and Reverses Hyperinsulinemia-Induced Hypertension in Rats

Wann-Chu Huang, Te-Chao Fang, Juei-Tang Cheng

Abstract—Experiments were performed to evaluate the role of the renal nerves in hyperinsulinemia-induced hypertension. Male Sprague-Dawley rats were made hyperinsulinemic by insulin infusion via osmotic minipumps implanted subcutaneously (3.0 mU/kg per minute for 6 weeks). Rats with vehicle infusion served as controls. Bilateral renal denervation was performed either at the beginning of or 4 weeks after insulin infusion. The systolic blood pressure was measured by the tail-cuff method twice a week. Food and water intake and urine flow were measured daily. The results showed that sustained insulin infusion significantly increased plasma insulin concentrations from 277.7±25.8 pmol/L to 609.9±22.2 and 696.7±23.0 pmol/L by the end of weeks 4 and 6, respectively (P<0.05). Systolic blood pressure was significantly increased from 135±3 to 157±3 and 159±2 mm Hg (P<0.05) at the corresponding time points. There was a significant increase in the plasma norepinephrine concentration after insulin infusion, whereas no significant changes in plasma triglyceride and glucose concentrations, water intake, urine flow, sodium excretion, sodium gain, and body weight gain were observed. Bilateral renal denervation depleted renal norepinephrine stores and prevented the development of hyperinsulinemia-induced hypertension. After hyperinsulinemia-induced hypertension had been fully established (from 134±2 to 157±2 mm Hg), bilateral renal denervation reversed the elevated systolic blood pressure to normotensive levels within 2 weeks. Transient denervated diuresis and natriuresis were observed. These results indicate that chronic hyperinsulinemia-induced hypertension requires the presence of intact renal nerves in rats.

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Key Words: hyperinsulinemia ■ insulin resistance ■ renal nerve ■ renal denervation ■ denervated natriuresis

Numerous studies have provided strong inferential evidence that positively associates hypertension with insulin resistance and hyperinsulinemia in humans and some genetically hypertensive rats.1–6 Sustained high carbohydrate feeding in rats results in hypertension that is also correlated with insulin resistance and hyperinsulinemia.7–9 Moreover, we and others have demonstrated that long-term insulin administration causes hypertension in rats.10–13 The hyperinsulinemia-induced rise of blood pressure is reversible with termination of insulin infusion, thus denoting a specific effect of hyperinsulinemia.10 These observations provide direct support for an important role for hyperinsulinemia or a hyperinsulinemia-associated mechanism in causing hypertension. However, the precise mechanism coupling hyperinsulinemia to the development of hypertension is not yet clear. Some short-term studies showed that insulin could increase the renal reabsorption of sodium and reduce sodium excretion in animals and humans.14,15 Also, acute or chronic elevation in plasma insulin level stimulates the sympathetic nervous system and increases plasma catecholamines.16–18 It follows that hyperinsulinemia may exert a pressor effect by modification of plasma volume and/or sympathetic nerve activity if the stimulating actions of insulin on the kidney and sympathetic nervous system persist chronically and are of sufficient magnitude.

It is recognized that renal regulation of fluid and sodium balance plays a dominant role in the long-term control of arterial pressure in normal and pathophysiological conditions19 and that the renal nerves substantially control the kidney functions.20 Stimulation of the efferent renal nerves alters renal hemodynamics and enhances tubular reabsorption and renin secretion, whereas stimulation of afferent renal nerves results in activation of neurons in the central nervous system that are involved in cardiovascular regulation and renal function.20,21 Thus, it is likely that increased renal nerve activity is intimately implicated in the pathogenesis of hypertension. Indeed, complete renal denervation (RD), or renal afferent denervation, has been shown to abolish or attenuate some forms of genetic and experimental hypertension.22–28 An increase in renal sympathetic nerve activity has also been observed in obesity-induced hypertension accompanied by hyperinsulinemia and insulin resistance.28 It is unclear whether the renal nerve activity is increased in chronic hyperinsulinemia and thereby involved in the development of
hypertension in this state. In the present study, the potential role of the renal nerves in the pathogenesis of hypertension induced by sustained insulin infusion was assessed directly by observing the effect of bilateral RD on the subsequent genesis or maintenance of hypertension in insulin-infused rats. The results reveal that bilateral RD not only prevented the rise of blood pressure due to insulin infusion but also effectively reversed the already established hypertension produced by long-term insulin administration. Thus, the presence of intact renal nerves is essential for the pathogenesis of hypertension due to sustained hyperinsulinemia in rats.

Methods

Animal Groups
Male Sprague-Dawley rats with an initial body weight of 180 to 220 g were used for the study. All experimental procedures were carried out in accordance with the prior approval of the Institutional Animal Care and Use Committee of this school. Rats were housed in individual metabolism cages placed in the animal room with room temperature controlled at 22 ± 1°C, were maintained on a laboratory rat diet containing 0.31% sodium (TD 90365, Teklad Premier), and were provided tap water ad libitum. Rats were divided into 4 groups: group 1 was the control, which received vehicle infusion only (n=8); group 2 included rats that received insulin infusion alone (n=8); group 3 received insulin infusion and concurrent bilateral RD (n=8); and group 4 received insulin infusion and bilateral RD performed 4 weeks later (n=8).

Experimental Protocols

After the control period of 6 days, an osmotic minipump (No. 2002, 14 days of active life, Alza Corp) filled with either insulin (3.0 mU/kg per minute) or vehicle was implanted subcutaneously via subcutaneous osmotic minipump (3 mU/kg per minute) and was under anesthesia (60 mg/kg ketamine hydrochloride and 9 mg/kg xylazine IP). At the end of the life of the minipump, a new one was implanted and the used one removed. The residual volume in each removed minipump was carefully examined to make sure that the minipump release function had been working normally as claimed by the manufacturer. After installation of the osmotic minipump, rats were continued on the controlled-sodium diet throughout the experiments. After 6 weeks of sustained insulin infusion, the osmotic minipump was removed and insulin infusion was stopped. The body weight was measured twice a week. Food and water intake and urine output were measured daily. The systolic blood pressure (SBP) was measured twice a week by the tail-cuff method with a programmed electrophotometry (model 343, Instrumentation Laboratory). The sodium gain was computed as the difference between sodium intake and urinary sodium output.

Renal Denervation

Bilateral RD was performed on group 3 rats at the beginning of insulin infusion and on group 4 rats after 4 weeks of insulin infusion. The procedures for RD have been detailed previously. The procedures for RD have been detailed previously.30 In brief, rats were anesthetized (60 mg/kg ketamine hydrochloride and 9 mg/kg xylazine IP) and the kidneys exposed through a retroperitoneal flank incision. RD was accomplished by carefully stripping all visible renal nerves along the renal arteries and veins from the aorta to the hilum of the kidney. Both renal arteries and veins then were swabbed with a solution of 10% phenol in absolute alcohol for ~10 minutes.

Results

Figure 1 compares the blood pressure responses to vehicle administration and sustained insulin infusion alone or in combination with bilateral RD. The SBP did not change...
significantly throughout the experimental period in control rats that received vehicle infusion. In contrast, the SBP of rats that received insulin infusion alone significantly increased from 135 ± 3 to 145 ± 2 mm Hg (P < 0.05) within 3 days. The blood pressure further increased to 157 ± 3 mm Hg by the end of week 4, and thereafter elevated blood pressure was maintained until the end of the experiments (159 ± 2 mm Hg).

Sustained insulin infusion into rats with bilateral RD failed to increase their blood pressure. After insulin infusion–induced hypertension had been fully established (from 134 ± 2 to 157 ± 2 mm Hg after 4 weeks of insulin infusion), subsequent denervation of both kidneys reversed the elevated blood pressure to levels comparable with preinfusion levels (138 ± 2 mm Hg) within 2 weeks.

The effects of insulin infusion alone and of insulin combined with bilateral RD on food intake, water intake, urine flow, and sodium excretion are illustrated in Figure 2. There was no significant difference in food intake among groups during the entire experimental period. However, bilateral RD caused a transient polydipsia, diuresis, and natriuresis in rats with insulin infusion. The dipsogenic and renal effects of RD subsided within 1 week, and thereafter water intake and urinary excretion of water and sodium appeared to be not different from those of the other groups.

The changes in daily sodium gain in control rats and insulin-infused rats with or without bilateral renal denervation are shown in Figure 3. No significant alterations in daily sodium gain were noted in rats that received either vehicle or insulin infusion alone. Bilateral RD caused transient reductions in sodium gain for ≈1 week in insulin-infused rats (groups 3 and 4). Figure 4 depicts the changes in body weight gain. There were no significant differences in weekly body weight gain between control rats and insulin-infused rats with or without RD throughout the experiments.

The Table summarizes the changes in plasma concentrations of insulin, triglycerides, glucose, and catecholamines in rats that received insulin administration alone and insulin combined with bilateral RD. The plasma insulin concentrations were approximately doubled after 4 weeks of insulin infusion and remained elevated until the end of the experiments. RD did not alter the plasma insulin concentration. There were no significant changes in the plasma levels of triglycerides, glucose, and epinephrine throughout the experiments in rats with and without insulin administration. Insulin infusion alone for 4 weeks (group 2) increased plasma NE levels, and these elevated plasma NE levels were maintained until the end of the experiments. However, no significant increase in plasma NE concentrations were observed in rats with combined insulin infusion and bilateral RD (group 3). In group 4, insulin infusion produced a significant increase in the plasma NE concentrations before RD. Two weeks after bilateral RD, the plasma NE concentrations significantly decreased to control levels.

Figure 2. Effects of insulin infusion alone and insulin combined with bilateral renal denervation on food intake, water intake, urine flow, and sodium excretion. Symbols, animal numbers, and statistical notations are defined in Figure 1.

Figure 3. Changes in daily sodium gain in control rats and insulin-infused rats with or without bilateral renal denervation. Symbols, animal numbers, and statistical notations are defined in Figure 1.
Renal tissue NE concentration was determined after completion of the experimental protocol in control and insulin-infused rats with and without bilateral RD. Sustained insulin infusion for 6 weeks did not change the renal NE contents (29.4±1.6 pmol/mg for control rats versus 32.3±2.1 pmol/mg for insulin-infused rats, \( P=0.1 \)). However, bilateral RD performed at either the beginning of (group 3) or 4 weeks after (group 4) insulin infusion depleted renal tissue NE stores by 94% (the residual NE contents were 2.1±0.5 pmol/mg for group 3 and 2.7±0.4 pmol/mg for group 4).

**Discussion**

The present study demonstrates that long-term administration of insulin resulted in sustained hypertension in rats. The hyperinsulinemia-induced increases in blood pressure occurred as early as the first week of insulin infusion, and the elevated blood pressure was maintained during insulin infusion. These observations confirm the previous studies from this and other laboratories and support the notion that euglycemic hyperinsulinemia causes hypertension in rats. In addition, this study reveals an important role for the renal nerves in the pathogenesis of hypertension associated with hyperinsulinemia. As shown in Figure 1, bilateral RD effectively prevented the elevation of blood pressure due to insulin infusion. After hyperinsulinemia-induced hypertension had been fully established, subsequent denervation of both kidneys precipitously reduced the blood pressure, which returned to normotensive levels within 2 weeks. Our present results indicate that the integrity of the renal nerves is essential for the initiation and maintenance of this type of hypertension in rats.

The dependency of the pathogenesis of hypertension on intact renal nerves is not unique for the hyperinsulinemic rat model. It has been demonstrated that renal denervation prevents or attenuates some forms of genetic and experimental hypertension in animals such as spontaneously hypertensive rats, New Zealand genetically hypertensive rats,24 2-kidney models of Goldblatt hypertensive rats,25 low-sodium, 1-kidney hypertensive rats,26 angiotensin-induced hypertensive rats,27 and obesity-induced hypertensive dogs,28 although negative results were reported in Dahl salt-sensitive rats31 and Lyon hypertensive rats32 and conflicting results were also obtained in hypertensive models induced by aortic coarctation.33,34 NO synthase inhibition,35,36 and deoxycorticosterone acetate salt treatment37,38 and in the 1-kidney model of Goldblatt hypertensive rats.39,40 In the present study, bilateral RD depleted the renal tissue NE content by 94% (measured at the end of the experiments, ie, 2 to 4 weeks after RD), suggesting that the denervation procedure was effective and that significant reinnervation had not yet occurred. It is known that the kidney possesses both efferent sympatheic and afferent sensory innervation. The efferent renal nerves may increase blood pressure by stimulating renin secretion or by causing sodium retention through direct and indirect actions on renal tubular reabsorption.20,21 The afferent renal
nerves may elevate arterial pressure via a centrally mediated mechanism to increase sympathetic nerve activity, resulting in increased blood pressure and peripheral resistance. The latter is evidenced by the observations that selective afferent renal denervation reduces central sympathetic neurotransmitter stores and attenuates hypertension in rats of 1-kidney, 1-clip and aortic nerve transection models.

The mechanism by which bilateral RD exerts its protective action against the development of hypertension or its antihypertensive effect in the already established hypertension in hyperinsulinemic rats is unclear. The denervation procedures in the present study interrupted both the efferent and afferent renal nerve fibers and therefore cannot define the contribution of each neural pathway to the pathogenesis of hypertension. Further studies are needed to differentiate which neural traffic is responsible for this effect and to determine the quantitative importance of these neural pathways in contributing to the development of hyperinsulinemia-induced hypertension.

In addition to causing a pressor effect, acute or chronic hyperinsulinemia has been reported to enhance sympathetic nerve activity in rats. Thus, it has been hypothesized that hyperinsulinemia exerts a hypertensive effect, at least partly by activating the sympathetic nervous system. We demonstrated previously that neonatal chemical sympathectomy delayed and attenuated the subsequent insulin-induced elevations of blood pressure in rats. Some other studies also demonstrated that the hypertensive response to hyperinsulinemia was attenuated by administrations of an α1-receptor antagonist or an α2-agonist, suggesting a modulatory role for the sympathetic nervous system in hyperinsulinemia-induced hypertension. However, there is a possibility that sympathetic activation may be a consequence of hyperinsulinemia-induced hypoglycemia as seen in a previous study, and hence the compensatory increase in sympathetic nerve activity during insulin infusion may be responsible for the rise in blood pressure. In accordance with this contention is the finding that chronic adrenergic receptor blockade with propranolol and prazosin did not prevent hyperinsulinemia-induced hypertension when euglycemia was maintained by continuous intravenous glucose infusion in rats. 

In the present study, the plasma NE concentration, an indirect marker of sympathetic nerve activity, increased significantly in insulin-infused rats (Table). Whether this was a response secondary to insulin-induced hypoglycemia or a direct effect of insulin per se is unclear because the blood glucose level was measured 28 days after the insulin infusion was begun. Nevertheless, prior RD prevented insulin infusion–induced hypertension, suggesting an important role for the renal nerves in the pathogenesis of hyperinsulinemia-induced hypertension. Bilateral RD also resulted in a significant decrease in plasma NE levels. We speculate that this could be due to an interruption of the afferent renal nerve activity that, by a direct feedback mechanism, attenuated systemic sympathetic tone. Consistent with this hypothesis is the observation that afferent sympathetic signals from the kidney play an important role in modulating efferent sympathetic responses. It is worth noting, however, that the elevated plasma levels of insulin, triglycerides, and glucose of hyperinsulinemic, hypertensive rats were not altered after denervation of both kidneys, whereas blood pressure was reduced significantly. This suggest that probably not hyperinsulinemia per se but a hyperinsulinemia-associated mechanism is responsible for the pathogenesis and maintenance of hypertension under these experimental conditions. It needs to be noted that the hypertensive action of hyperinsulinemia appears to be species specific, because it dose not occur in dogs. Thus, there is a diversity in the contribution of sustained hyperinsulinemia and its associated neural effects to the pathogenesis of hypertension in these experimental models. Whether this variety reflects the extent to which the sympathetic nervous system is stimulated in these various models and whether this is a result of species difference is unclear.

Acute administration of insulin has been shown to increase renal tubular reabsorption of sodium and water. If the acute antinatriuretic and antidiuretic effects of insulin can persist in a chronic setting such as the present study and are of sufficient magnitude, the resultant sodium retention with subsequent extracellular volume expansion may be a potential mechanism for hypertension in the hyperinsulinemic state. In fact, we demonstrated previously that there were no significant differences in urine flow, urinary sodium excretion, sodium accumulation, and body weight gain between rats with and without insulin infusion. Similar renal response patterns were observed in the current experiments (Figures 2 through 4) and in other studies. Furthermore, bilateral RD caused only transient diuresis and natriuresis, and thereafter no significant differences in the urinary excretion of water and sodium, sodium gain, and body weight gain between insulin-treated rats with and without renal innervation were noted. These observations imply that the hyperinsulinemia-associated increase in blood pressure is unrelated to obesity and that increased sodium retention due to enhanced renal nerve activity did not occur in insulin-treated rats. Thus, the inability of hyperinsulinemia to increase blood pressure after bilateral RD and the depressor effect of bilateral RD in hyperinsulinemia-induced hypertension were not mediated by alterations in sodium intake or excretion, water intake or excretion, or both. This notion is further supported by the finding that a high sodium diet did not aggravate the hypertension, and a low sodium diet did not alleviate the hypertension in insulin-infused rats. On the other hand, the observation that significant increases in blood pressure did not accompany an increased sodium excretion rate in insulin-infused rats suggests that these hypertensive rats had impaired pressure natriuresis, which was shifted to the right and reset at higher pressure levels.

In summary, the present study demonstrates that sustained infusion of insulin significantly increases plasma NE levels and blood pressure in normal rats. Despite the elevation in blood pressure, there were no significant differences in urine flow, sodium excretion, sodium accumulation, and body weight gain between rats with and without insulin infusion. Bilateral RD depleted renal tissue NE stores, reduced plasma NE concentrations, and prevented the rise of blood pressure due to sustained insulin infusion. When hyperinsulinemia-induced hypertension had been fully established, subsequent denervation of both kidneys rapidly reversed the elevated blood pressures to normotensive levels. These results suggest
that sustained hyperinsulinemia causes hypertension, which is renal nerve dependent in rats.

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