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Role of the Sympathetic Nervous System During the Development of Obesity-induced Hypertension in Rabbits

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We have previously reported that weight gain induced by high-fat diet (HFD) leads to an increase in mean arterial pressure (MAP, +14%) and heart rate (HR, +31%) in the adult rabbit. In the present study, we tested the hypothesis that an increased activity of the sympathetic nervous system may contribute to the development of obesity-induced hypertension. A combination of α- and β-adrenergic blockers (terazosin + propranolol) was chronically administered to rabbits housed in metabolic cages for continuous monitoring of arterial pressure by telemetry, 24 h a day. After 2 weeks of adrenergic blockade under control diet, animals were switched to HFD for the next 6 weeks. HFD induced a progressive increase in body weight, but no increase in mean arterial pressure (+0.2 ± 2.5%) and a slight increase in heart rate (+14 ± 3%). Time-control animals fed normal diet showed no changes in MAP or HR with long-term α- and β-adrenergic blockade. Our results indicate that the activation of the sympathetic nervous system may play an important role in the pathogenesis of obesity-induced hypertension. Am J Hypertens 2000;13:556–559

KEY WORDS: **Obesity, hypertension, sympathetic blockade, rabbits, high-fat diet.**

The association between obesity and hypertension has been recognized for many decades. However, the basic physiologic mechanisms that link body weight and blood pressure are still incompletely understood. Several lines of evidence indicate that the activity of the sympathetic nervous system may be elevated in obesity. First, a correlation between blood pressure and norepinephrine levels has been shown in borderline obese hypertensive subjects and during blood pressure reduction induced by weight loss.[1] Second, increased caloric intake activates the sympathetic nervous system, whereas fasting reduces sympathetic activity.[2] Third, in healthy humans, body fat is a major determinant of the resting rate of muscle sympathetic nerve discharge.[3] Finally, studies in dogs[4,5] support the importance of sympathetic activation during rapid weight gain as a plausible mechanism contributing to the increased blood pressure.

Adult rabbits fed an ad libitum high-fat diet (HFD) represent an attractive and promising model to study the mechanisms of obesity-induced hypertension. These rabbits exhibit hemodynamic and neurohumoral changes that mimic changes observed in human obesity, including hypertension, a hyperdynamic

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circulation with increased heart rate and cardiac output, elevations in plasma insulin and plasma renin activity, cardiac hypertrophy, and impaired diastolic function, as reported previously.[6,7]

Recently, we have studied the sequential cardiovascular and renal changes that occur in rabbits during weight gain induced by a HFD.[8] HFD induced a rapid increase in mean arterial pressure, heart rate, and glomerular filtration rate that stabilized, respectively, at 14%, 31%, and 68% above control values. The model was also characterized by a net accumulation of sodium, with significant sodium retention during the first 3 weeks of HFD. Time-course analysis of the changes in blood pressure in this model suggests that obesity-induced hypertension may consist of two phases: the early phase could be due to high-fat intake or overfeeding, whereas the later phase seems to be strongly related to weight gain and increased fat mass.

To test the hypothesis that an increased activity of the sympathetic system may play a role in both the early and later phases of obesity-induced hypertension, we studied freely moving rabbits during the development of obesity while sympathetic activity was blocked pharmacologically. To that purpose, we administered a combination of α- and β-adrenergic antagonists, to block the peripheral vasoconstrictor actions and direct renal tubular effects of sympathetic activation, and to prevent the sympathetically mediated stimulation of the heart and of renin release.

MATERIALS AND METHODS

Animal Preparation Adult male lop-eared rabbits (body weight 4.33 ± 0.10 kg) of the breed "Bélier Français" were used in this study. All protocols were approved by the State Animal Committee. Under halothane anesthesia and aseptic techniques, the rabbits were instrumented with an implantable pressure transducer and radiotransmitter (model TA11PA-C40, Data Sciences International, St. Paul, MN) to monitor arterial pressure by telemetry, as described previously.[8] After surgery, the rabbits were housed in individual cages, customized for urine collection, in a quiet air-conditioned room with a 12-h light–dark cycle. The rabbits were allowed to recover for at least 10 days before the start of the experiments. All animals had free access to tap water and were fed 180 g of standard rabbit chow per day.

Continuous Hemodynamic Monitoring by Telemetry Each metabolic cage was equipped with receivers, connected by a multiplexer to a calibrated pressure analog adapter, as described previously.[8] The analog pressure signal was then sent to an A/D converter and processed by a personal computer using customized algorithms[9] to compute mean arterial pressure (MAP) and heart rate (HR) on a beat-to-beat basis. The signal was sampled at 500 Hz, for 5-sec periods every 30 sec, from 10:00 AM to 8:00 AM the next morning. Weekly results are reported as the average of 7 consecutive days.

Experimental Protocol After 1 week of control measurements rabbits were given a combination of α- and β-adrenergic blockers for the next 8 weeks. Terazosin monohydrochloride (Abbott Laboratories, Abbott Park, IL) and DL-propranolol (Sigma, St. Louis, MO) were added to the drinking water and the concentration was adjusted according to the average water intake of the animals to assure a daily drug intake of ≈20 mg/kg of each substance. To test the adequacy of the sympathetic blockade, we analyzed the cardiovascular response to the intravenous injection (through an ear vein) of an α-adrenergic agonist (phenylephrine 16 μg/kg) or a β-adrenergic agonist (isoproterenol 0.05 μg/kg) before and during α and β blockade.

After 2 weeks of sympathetic blockade, the rabbits (n = 5) were switched to a HFD ad libitum (standard rabbit chow with 10% added fat as soya oil-to-pork fat 1:1) for another 6 weeks. Three additional rabbits were treated with adrenergic blockers for 8 weeks, but were kept on the standard rabbit chow to serve as time-controls. Water intake, food intake, and 24-h urine output were determined daily at ≈9:00 AM. Body weight was measured twice a week. Blood samples were withdrawn weekly by arterial puncture of the central ear artery for various analytic measurements.

Analytical Measurements Plasma and urine sodium and potassium were determined by flame photometry (model IL 943, Instruments Laboratoires, Lexington, MA), and creatinine by the kinetics of Jaffé (Beckman creatinine analyzer 2, Fullerton, CA). Glomerular filtration rate (GFR) was assessed based on creatinine clearance. Sodium balance was calculated as the difference between sodium intake and urinary sodium excretion, taking into account an average of 20% nonrenal (fecal) loss of sodium (results from separate experiments), consistent with values reported by Grace et al.[10]

Statistical Analysis Statistical analysis within group was performed by analysis of variance for repeated measurements. Because each rabbit within a group serves as its own control, experimental values were compared with control values in each group using Dunnett's multiple comparison procedure. Changes were considered to be statistically significant if $P < .05$.

RESULTS

In the five pre-HFD rabbits, combined α and β blockade resulted in an immediate decrease in MAP from 66.1 ± 2.1 mm Hg to 59.3 ± 2.0 mm Hg (−11.2 ± 2.0%) on day 1, but MAP increased slowly over the next few days to plateau at 63.9 ± 1.9 mm Hg (−3.3 ± 2.0%) during the second week of pharmacologic blockade. Sympathetic

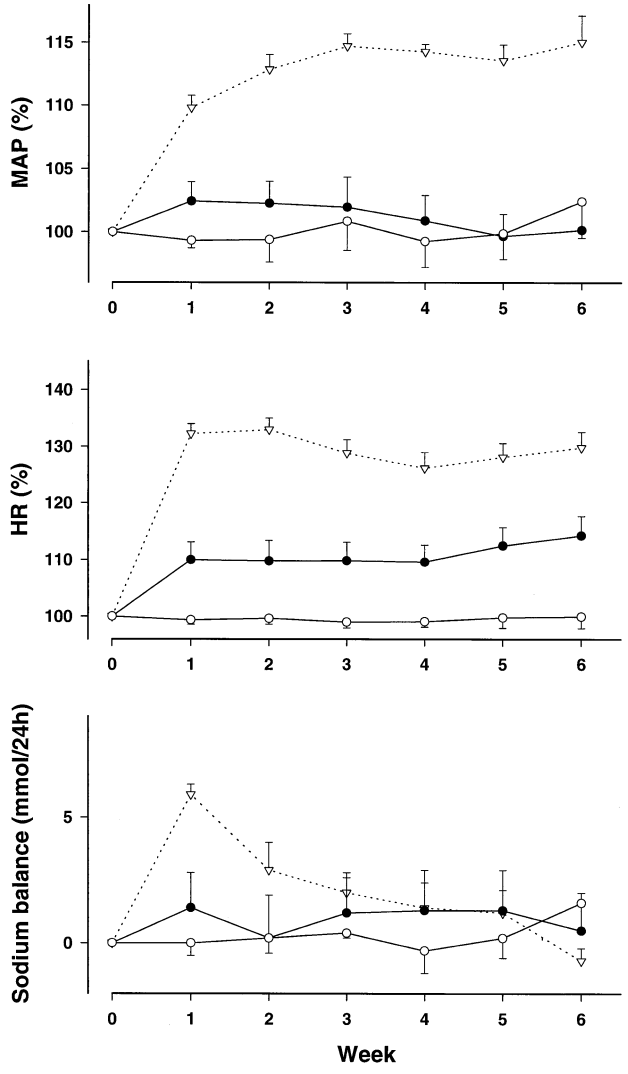

FIGURE 1. *Percent changes in mean arterial pressure (MAP), heart rate (HR), and sodium balance in rabbits submitted to 6 weeks of high-fat diet (solid line, closed symbols) or in time-control rabbits (solid line, open symbols) during α + β-adrenergic blockade. Average sodium balance during the control period was subtracted from the subsequent weekly averages. Values are expressed as mean ± SE. The dotted line shows the changes observed in high-fat diet animals without sympathetic blockade (from our previous study[8]).*

blockade induced the same changes in MAP in time-control rabbits (n = 3). The adequacy of sympathetic blockade was assessed by pharmacologic tests. Before blockade, intravenous phenylephrine (16 μg/kg) increased MAP by 37.8 ± 0.5 mm Hg, whereas intravenous isoproterenol (0.05 μg/kg) increased HR by 71.4 ± 7.2 beats/min. During long-term blockade, the increases in MAP and HR induced by phenylephrine and isoproterenol were only 13.2 ± 1.9 mm Hg and 8.8 ± 3.1 beats/min, respectively.

Figure 1 illustrates the sequential changes in MAP, HR, and sodium balance during 6 weeks of HFD diet (n = 5) or normal diet (n = 3) during sympathetic blockade. In Figure 1, the control week (week 0, 100%) corresponds to the second week of pharmacologic blockade, with values of MAP and HR of 63.9 ± 1.9 mm Hg and 195 ± 5 beats/min in the HFD group (n = 5), and 59.3 ± 1.1 mm Hg and 182 ± 6 beats/min in the time-control group (n = 3). Despite a progressive increase in body weight from 4.46 ± 0.12 kg to 5.41 ± 0.23 kg in HFD rabbits, MAP remained unchanged throughout the pharmacologic blockade (63.8 ± 1.3 mm Hg at week 6 of HFD, or 0.2 ± 2.5% above control). In contrast, HR increased immediately with HFD (214 ± 4 beats/min or +10 ± 3%, week 1) and remained elevated throughout HFD (222 ± 8 beats/min or +14 ± 3%, week 6). In time-control rabbits fed a normal diet, MAP and HR remained stable throughout the experimental protocol (60.6 ± 0.6 mm Hg and 182 ± 8 beats/min, respectively, during the last experimental week). The changes in MAP, HR, and sodium balance from our previous study[8] (intact rabbits without sympathetic blockade, fed an HFD) are included in Figure 1 for comparison.

Two weeks of combined α and β blockade increased GFR from 10.2 ± 0.8 to 12.2 ± 1.3 mL/min (+18.4 ± 4.1%), but this change did not reach statistical significance (n = 5). Similar changes were observed in time-control rabbits, from 9.8 ± 0.4 mLmin to 11.7 ± 0.8 mL/min (+18.4 ± 7.2%). However, when data of both groups were pooled (n = 8), the increase in GFR was significant after 2 weeks of blockade. In the HFD group, a further increase of GFR to 13.7 ± 0.8 mL/min was observed, but the change was only significant when compared to the period before blockade. GFR remained stable in our time-control rabbits (11.8 ± 1.7 mL/min). Finally, analysis of sodium balance in HFD rabbits showed a slight but statistically nonsignificant tendency for sodium retention with the HFD. In contrast, potassium balance data in HFD rabbits (computed as potassium intake minus urinary potassium excretion) showed potassium retention over the entire experimental period. Sodium and potassium balances remained stable in time-control rabbits.

DISCUSSION

The most significant finding of the present study is that pharmacologic blockade of the sympathetic nervous system with a selective α1-antagonist (terazosin) and a nonspecific β-receptor antagonist (propranolol) prevents the increase in MAP induced by a high-fat, high-calorie diet in rabbits. In our previous study,[8] we reported that rabbits fed an ad libitum HFD showed a rapid increase in MAP already after 3 days of HFD (+9%) that stabilized at 14% above control over the next 7 weeks. Sympathetic blockade appeared to block both the early and late increase in blood pressure, suggesting that an activation of the sympathetic nervous system is involved in both phases of obesity-induced hypertension.

Interestingly, the increased HR (+31%) induced by HFD alone[8] was not completely suppressed by sympathetic blockade, but simply attenuated (+14%). If one assumes effective β blockade as evidenced by our isoproterenol challenge tests, those data suggest that mechanisms other than cardiac sympathetic activation are partially responsible for the tachycardia observed in this model of hypertension. Possible mechanisms include a metabolically mediated increase in intrinsic HR or a reduction in vagal tone as has been observed in obese dogs.[11] Our renal data are also consistent with an overall sympathetic hyperactivity in obesity-induced hypertension. In the present study, sympathetic blockade markedly attenuated the sodium retention observed in rabbits during weight gain.[8] In the intact animals, an increase in plasma renin activity, as observed in obese dogs[12] and rabbits,[6] and direct tubular effects of sympathetic activation,[13] may both contribute to sodium retention. Therefore, inhibition of these mechanisms may explain the observed attenuation of sodium retention.

The marked increase in GFR by HFD alone[8] was also attenuated in the present study. As in other studies[12], we postulated that the increase in GFR in intact obese animal models could be related to an increased tubular reabsorption at a site proximal to the macula densa, resulting in a compensatory increase in GFR. Decreased tubular reabsorption induced by sympathetic blockade would thus minimize the increase in GFR. An alternate explanation is that sympathetic blockade alone increased GFR by 18%, which could have blunted a further increase in GFR with HFD.

Our study in rabbits is also in accordance with observations on the role of sympathetic nervous system in obese dogs. Combined adrenergic blockade for 7 days decreases blood pressure to a greater extent in obese dogs compared to lean dogs,[4] and bilateral renal denervation attenuates the sodium retention and hypertension associated with obesity.[14] Recently, Rocchini et al[5] have shown that clonidine, a central α_2 agonist that inhibits central nervous sympathetic activity, prevents insulin resistance and hypertension in obese dogs.

The mechanisms by which weight gain leads to sympathetic activation are not yet fully understood, but an interesting candidate is leptin. Leptin production correlates positively with body mass index and chronic leptin infusion increases both blood pressure and heart rate in rats,[15] probably through sympathetic activation.[16] In addition, leptin is unable to increase blood pressure and HR in rats during combined α and β blockade.[17] In summary, our study suggests an important role of the sympathetic nervous system during the development of obesity-induced hypertension.

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