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Dietary salt loading exacerbates the increase in sympathetic nerve activity caused by intravenous insulin infusion in rats

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Abstract

Obesity and type 2 diabetes mellitus frequently produce chronic elevations in blood insulin levels. Importantly, hyperinsulinemia stimulates increases in sympathetic nerve activity that may predispose to hypertension, atherosclerosis, and end-organ damage. Because depletion of dietary salt (NaCl) increases angiotensin II levels, which has been shown to enhance sympathetic responses to excitatory stimuli such as thermal stimulation and bicuculline in the hypothalamus, we predicted that insulin-induced elevations in lumbar sympathetic activity would be augmented by low NaCl and suppressed by high dietary NaCl. Adult male Sprague-Dawley rats were randomized into groups receiving low (0.0 mEq/d, n = 10), normal (2.0 mEq/d, n = 10), and high (5.7 mEq/d, n = 10) NaCl for a period of 8 days. After this, the animals were anesthetized for measurement of heart rate, mean arterial pressure, and lumbar sympathetic nerve activity during 110 minutes of intravenous insulin infusion (15 mU/kg per minute) with euglycemic clamp. Insulin administration caused modest blood pressure decreases accompanied by heart rate increases that were similar across the 3 dietary groups. Unexpectedly, sympathetic increases to insulin were lowest in the low-NaCl group (100%-135% ± 24%), moderate in the normal-NaCl group (100%-170% ± 23%), and greatest in the high-NaCl group (100%-252% ± 39%). Dietary NaCl level did not affect baseline blood glucose or insulin sensitivity as assessed by euglycemic clamp. These findings indicate that dietary salt loading exacerbates the lumbar sympathoexcitatory response to intravenous insulin infusion in rats.

1. Introduction

There is now abundant evidence that elevations in blood insulin levels cause activation of the sympathetic nervous system. In short-term studies using experimental animals [1-6], both systemic and intracerebroventricular (ICV) infusion of insulin provokes elevations in renal, adrenal, brown adipose tissue and lumbar sympathetic nerve activity (SNA), whereas in humans, short-term insulin activates muscle SNA without affecting SNA in skin [7-10]. In long-term studies, osmotic minipump administration of insulin in rats generates long-term increases in plasma norepinephrine, heart rate (HR), and blood pressure [11-13]. Importantly, long-term elevations in SNA have been shown to exert several pathophysiologic effects on the cardiovascular system, including hypertension, atherosclerosis, left ventricular hypertrophy, insulin resistance, and end-organ damage [14-16].

Although it is not known how insulin stimulates SNA, recent observations point to an important role for angiotensin II. For example, in rats, infusion of insulin produced elevations in blood pressure, HR, and plasma norepinephrine that were abolished by converting enzyme inhibition or by angiotensin II–type AT_1 receptor antagonists [11,12,17,18]. In our laboratory, euglycemic hyperinsulinemia caused elevations in lumbar SNA that were eliminated by converting enzyme inhibition with captopril [4], suggesting that insulin may activate components in the renin-angiotensin system to produce increases in sympathetic neural outflow.

Given this dependence of sympthoactivation on the renin-angiotensin system, it is possible that physiologic increases in endogenous angiotensin II provoked by dietary salt depletion would enhance sympathoexcitation caused by insulin. In favor of this, DiBona and Jones [19,20] reported that paraventricular injection of bicuculline and thermal-receptor stimulation both caused increases in renal SNA that were suppressed by dietary-salt loading and enhanced by dietary-salt depletion. In addition, congestive heart failure in

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rats, which greatly elevated plasma renin activity, also potentiated the increases in renal SNA to bicuculline [20]. These observations raise the possibility that lumbar sympathoexcitatory responses to insulin would be enhanced in rats on a low dietary sodium intake compared with rats on a normal or high dietary sodium intake. To test this, we administered low-, normal-, and high-NaCl regimens to adult rats for a period of 8 days and then infused insulin with euglycemic clamp while measuring HR, mean arterial pressure (MAP), and lumbar SNA, which is almost entirely postganglionic in composition at the L3 through the L5 level [1].

2. Methods

2.1. Animals

Male Sprague-Dawley rats, weighing 200 to 225 g, were purchased from Harlan (Indianapolis, IN). The rats were housed in a temperature-controlled colony room illuminated on a 12:12 light-dark cycle. All procedures were performed in accordance with the Lehman College Institutional Animal Care and Use Committee and the National Institutes of Health guidelines for the care and use of experimental animals.

2.2. NaCl regimens

At 1 week after arrival, the rats were divided into 3 dietary groups. All 3 groups were given nominally sodium-free pellet food ad libitum (MP Biomedicals, Solon, OH; <0.9 μEq/g sodium). The low-NaCl group (low NaCl, n = 10) received sodium-free distilled water as drinking fluid, the normal-sodium group (normal NaCl, n = 10) received 50 mmol/L NaCl as drinking fluid, and the high-sodium group (high NaCl, n = 10) received 154 mmol/L NaCl as drinking fluid. The rats equilibrated on these different dietary sodium intakes for a period of 8 days. On average, the low-NaCl group drank 27.9 ± 2.2 mL of fluid per day, which equates to a sodium intake of 0.0 mEq/d; the normal-NaCl group drank 40.9 ± 4.9 mL/d, which equates to a sodium intake of 2.0 ± 0.2 mEq/d; and the high-NaCl group drank 37.3 ± 3.9 mL/d, which equates to a sodium intake of 5.7 ± 0.6 mEq/d. The 2.0 mEq/d sodium intake in the normal-NaCl group is equivalent to the sodium intake of adult rats consuming normal chow (0.5%-1.0% NaCl) ad libitum [21]. In previous studies, this dietary-sodium treatment protocol significantly increased plasma renin activity in the low-NaCl group and significantly decreased plasma renin activity in the high-NaCl group when compared with the normal-NaCl group [20,22].

2.3. Surgical procedure

At the end of the diet period, the animals were anesthetized and prepared for cardiovascular monitoring during euglycemic insulin infusion. Anesthesia was induced with Brevital (40 mg/kg, IP, Henry Schein, Mellville, NY) and was maintained with urethane (0.8 g/kg, IV, Sigma, St. Louis, MO), and body temperature was kept near 37.5°C using a temperature-controlled surgical table and a heating lamp. The trachea was cannulated for spontaneous respiration of room air to prevent upper respiratory tract obstruction and hypoxia. Blood pressure was measured from a catheter in the left femoral artery using a pressure transducer (Statham P23XL, Astro-Med, West Warick, RI) connected to a PowerLab data acquisition system and a Macintosh computer. Heart rate was calculated from the blood pressure pulse using the PowerLab system. Two catheters were inserted into the left femoral vein for infusion of insulin and glucose. A final catheter was inserted into the tail artery to obtain samples for blood glucose, blood gases, and chemistries. Multifiber recordings of lumbar SNA were obtained as previously described [3]. Briefly, a midline abdominal incision was made, and a lumbar sympathetic nerve was isolated. The cut central end of the nerve was placed on a bipolar platinum electrode (Cooner Wire, Chatsworth, CA) and covered with dental impression material (Bisico S4, Bielfeld, Germany). Nerve activity was led through a Grass model HIP511 high-impedance probe (Astro-Med Grass, West Warwick, RI), amplified (×10 000-×20 000), and filtered (30-3000 Hz) with a Grass preamplifier (Model P511), and led through an oscilloscope (Model 54600A, Hewlett-Packard, Colorado Springs, CO), an audio monitor (Grass model AM8), and an integrator (Grass model 7P3) for display on the PowerLab system. The time constant for the 7P3 was set at 0.02 seconds, and rectification was set so that both positive and negative signals were integrated. To ensure that electrical noise was excluded from the assessment of sympathetic outflow, lumbar SNA was corrected for postmortem background activity.

2.4. Experimental procedure

During surgical preparation, all rats received saline through the venous catheter at a rate of 0.3 mL/h. After surgery completion, the rats were allowed to stabilize for 60 minutes before the experimental protocol. After this, basal levels of MAP, HR, lumbar SNA, and blood glucose were recorded during a 20-minute baseline period. In addition, a blood sample (125 μL) was drawn from the caudal artery into a preheparinized capillary tube for measurement of blood gases and chemistries using a blood gas analyzer (ABL-555, Radiometer, Copenhagen, Denmark). After the end of baseline, all rats received regular pork insulin (Iletin, Eli Lilly, Indianapolis, IN) in isotonic saline through the femoral vein using an infusion pump (Model 11, Harvard Apparatus, Hollitson, MA) at a variable rate to obtain a dose of 15 mU/kg per minute for a period of 110 minutes. Arterial blood glucose levels were measured from 1 drop of blood every 5 minutes using a portable glucometer (Accu-Check Advantage, Boehinger Mannheim, Indianapolis, IN). To maintain baseline blood glucose, or

Table 1
Effects of euglycemic hyperinsulinemia on plasma chemistries, blood gases, and glucose infusion rates in rats placed on NaCl diets for a period of 8 days

Measure	Low NaCl (n = 10)		Normal NaCl (n = 10)		High NaCl (n = 10)	
	Baseline	End infusion	Baseline	End infusion	Baseline	End infusion
Plasma potassium (mmol/L)	4.28 ± 0.07	3.42 ± 0.12*	4.20 ± 0.07	3.43 ± 0.54*	4.08 ± 0.08	3.23 ± 0.07*
Plasma sodium (mmol/L)	138.7 ± 0.8	140.7 ± 1.0*	140.1 ± 0.4	142.6 ± 0.3*	140.4 ± 0.3†	141.8 ± 1.1*
Plasma ionized calcium (mmol/L)	0.94 ± 0.05	0.97 ± 0.05	1.04 ± 0.03	1.04 ± 0.02	1.02 ± 0.02	1.04 ± 0.02
Hematocrit (%)	48 ± 2	43 ± 1	46 ± 2	40 ± 1	45 ± 1	46 ± 3
pH	7.45 ± 0.01	7.48 ± 0.01*	7.42 ± 0.01†	7.48 ± 0.01*	7.42 ± 0.01†	7.47 ± 0.01*
Arterial CO_2 (mm Hg)	39.1 ± 0.7	37.6 ± 0.9*	39.3 ± 0.8	38.4 ± 0.9*	40.0 ± 0.4	38.2 ± 0.4*
Arterial O_2 (mm Hg)	91.6 ± 4.0	99.9 ± 3.1*	88.6 ± 1.3	97.5 ± 1.0*	91.2 ± 4.0	103.3 ± 4.9*
Blood glucose (mg/dL)	80.5 ± 3.4	79.1 ± 2.5	75.0 ± 3.6	71.6 ± 4.7	78.2 ± 5.0	77.1 ± 3.8
Glucose infusion rate (mg kg^{-1} min^{-1})	–	32.5 ± 1.4	–	29.5 ± 1.3	–	31.0 ± 1.1

All values are means ± SEM. For blood glucose and glucose infusion rate, the depicted values represent data averaged over the final 30 minutes of infusion.
* $P < .05$ compared with baseline within the corresponding diet group.
† *$P < .05$ compared with low-NaCl baseline.*

euglycemia, a 50% glucose solution (in sterile water) was infused at variable rates using an infusion pump (Model 22, Harvard Apparatus). Mean arterial pressure, HR, and lumbar SNA were recorded continuously, and second blood-gas and chemistry samples were taken at the end of the 110-minute infusion period.

2.5. Statistical analysis

Basal MAP, HR, and lumbar SNA values were collected at 40 samples per second and then averaged over 20 minutes to obtain a single value for the baseline period. During the subsequent 110-minute experimental period, averages of 1-minute samples of MAP, HR, and lumbar SNA were obtained every 5 minutes. All data were analyzed using appropriate single or repeated-measures analysis of variance and presented as means ± SEM. Post hoc comparisons were made using Fisher least significant difference tests when the global F ratio was significant. Differences between groups were considered significant at the $P < .05$ level.

3. Results

3.1. Baseline data

The NaCl diets had no effect on plasma chemistries or blood gases before insulin infusion except for significantly lower plasma sodium in the low-NaCl compared with the high-NaCl group ($P < .05$) and lower pH in the low-NaCl group than in the other 2 groups ($P < .05$, Table 1). Body weights increased during the 8-day dietary regimen (low NaCl, 246 ± 10 to 268 ± 10 g; medium NaCl, 244 ± 12 to 277 ± 10 g; high NaCl, 236 ± 9 to 268 ± 8 g; $P < .0001$), and the NaCl diets had no effect on the rate of body weight increase. Baseline MAP and HR did not differ among the 3 dietary groups (Figs. 1 and 2, time = 0 minute). Means of lumbar SNA, measured during the

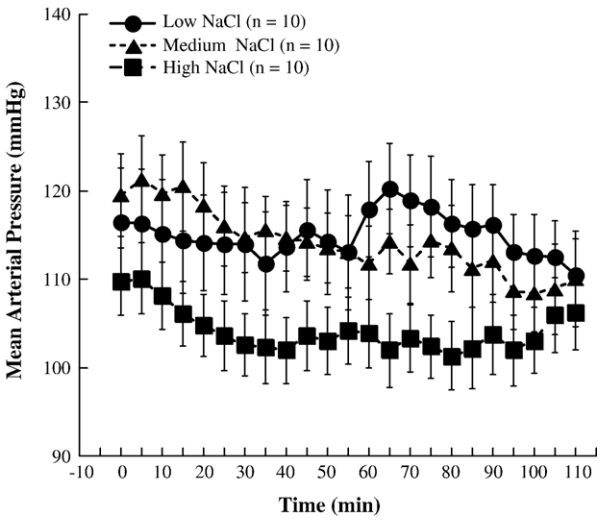

Fig. 1. Changes in mean arterial blood pressure from baseline (time = 0 minute) to a 110-minute infusion of insulin/glucose in rats that had been maintained on low-NaCl, normal-NaCl, or high-NaCl diets for 8 days. Values are means ± SEM.

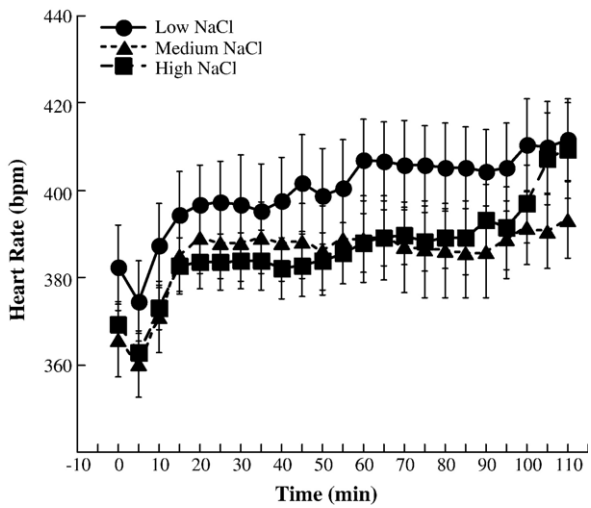

Fig. 2. Changes in heart rate from baseline (time = 0 minute) to 110 minutes of infusion of insulin/glucose in the same experimental groups. Values are means ± SEM.

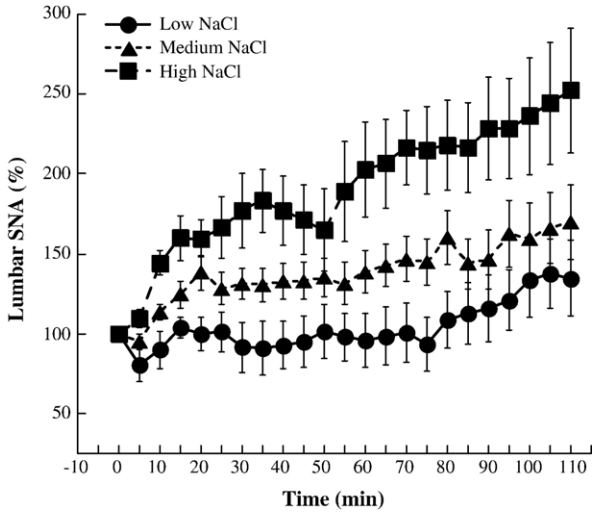

Fig. 3. Changes in lumbar SNA to 110 minutes of insulin/glucose in the same experimental groups. The 20-minute lumbar SNA baseline data (see Results) were taken as 100%, and lumbar SNA responses to insulin/glucose were expressed as percentage of this baseline level. Values are means ± SEM.

baseline period, were not different between the low-NaCl (46 ± 4 μV), normal-NaCl (46 ± 3 μV), and high-NaCl (48 ± 4 μV) groups. Therefore, lumbar SNA during the baseline period (Fig. 3, time = 0 minute) was taken as 100%, and the subsequent values were expressed as a percentage of this baseline level.

3.2. Responses to eulgycemic hyperinsulinemia

Plasma chemistries, blood gases, and glucose infusion rates at the end of insulin infusion are shown in Table 1. Insulin administration resulted in significant decreases in plasma potassium ($P < .001$) accompanied by increases in plasma sodium ($P < .0001$) that did not differ among the 3 dietary groups. The insulin infusion protocol was also associated with significant increases in arterial oxygen tension (Po_2, $P < .001$) and decreases in arterial carbon dioxide tension (Pco_2, $P < .001$). The decline in P_{CO2} was accompanied by an increase in pH ($P < .0001$). Again, these alterations were not different between the diet groups. Blood glucose remained stable during the infusion period, and the glucose infusion rate, indicating insulin sensitivity, was not different among the 3 dietary groups (Table 1).

Although insulin provoked variable MAP changes across the low-, medium-, and high-NaCl groups, all 3 dietary groups ended with pressures that were below their baseline levels (Fig. 1). These changes were reflected by a significant main effect for repeated measures ($P < .0001$) and a group by repeated-measures interaction ($P < .05$). For HR, insulin caused a brief bradycardia followed by a rapid HR increase, which stabilized into a long-lasting tachycardia. These alterations were similar in all 3 groups (Fig. 2). Analysis of variance of these data revealed a main effect for repeated measures ($P < .0001$), but no group by repeated-measures interaction. Finally, euglycemic hyperinsulinemia stimulated lumbar SNA increases that were smallest in the low-NaCl group, intermediate in the normal-NaCl group, and greatest in the high-NaCl group (Fig. 3). Analysis of variance of these data revealed a group by repeated-measures interaction ($P < .001$). Separate post hoc tests at each 5-minute interval indicated that the high-NaCl group had greater SNA than the low-NaCl group at all time points ($P < .05$) and that SNA in the high-NaCl group was greater than in the normal-NaCl group at 10, 15, 20, 35, 60, 65, 70, 75, 85, and 90 minutes into infusion ($P < .05$).

4. Discussion

The key finding of the present study is that increases in lumbar SNA due to euglycemic hyperinsulinemia were enhanced in rats on high dietary salt and practically abolished in rats on low NaCl. This pattern of findings was unexpected because others have reported the opposite, namely, that renal SNA responses to thermal stimulation and to paraventricular bicuculline were enhanced by low dietary salt and suppressed by high salt [19,20]. To explain this disparity, it is possible that the interaction between dietary salt and sympathetic reactivity may depend less on angiotensin II levels and more on the nature of the sympathoexcitatory stimulus. In support of this, previous work in humans showed that increases in muscle SNA due to nitroprusside infusion were attenuated in subjects on low dietary NaCl compared with the same subjects on regular NaCl [23,24]. Importantly, in the same experiments, increases in muscle SNA due to the cold pressor test were not affected by the level of dietary salt [23,24], indicating that the effect of sodium on SNA reactivity varies according to the nature of the stimulus. For the interaction with angiotensin II, the previous finding that captopril abolishes sympathetic activation due to hyperinsulinemia [4] suggests that insulin-mediated SNA increases depend more on the generation of new angiotensin II than on baseline angiotensin II levels.

The present finding that insulin-induced elevations in lumbar SNA were attenuated by low dietary NaCl may be explained by a baroreceptor mechanism. In agreement with previous studies [1,8], we found that acute insulin infusion produced modest decreases in MAP (Fig. 2), suggesting that part of the SNA increases may be secondary to unloading of the arterial baroreceptors. In support of this mechanism, prior experiments showed that blood pressure decreases elicited by sodium nitroprusside infusion resulted in muscle SNA increases that were attenuated in human subjects maintained on reduced-sodium diets compared with those on medium NaCl [23-25]. Thus, in the current study, decreasing sympathetic activation in the lower dietary NaCl groups may have been secondary to low salt-induced attenuation of SNA responses to baroreceptor stimulation. However, arguing against a baroreceptor mechanism are several studies showing a dissociation between the sym-

pathoexcitatory and vasodilatory actions of insulin [7,9,10]. In fact, some studies have shown insulin-induced increases in muscle SNA despite no change in MAP, forearm vascular resistance, or central venous pressure [10], or even during increases in forearm vascular resistance in elderly normotensive subjects [9]. More important in the context of the present experiments, increases in lumbar SNA due to euglycemic insulin infusion were nearly intact in rats subjected to sinoaortic denervation, indicating that the sympathoactivation was largely independent of the baroreflex [1].

If the lumbar SNA increases are not reflexogenic, they may result from a direct action of insulin on the central nervous system (CNS). In support of a central neural action, we and others have demonstrated that ICV administration of insulin raises SNA [3,6], whereas ablation of the hypothalamic anteroventral third ventricle region prevents increases in lumbar SNA to intravenous insulin infusion [2]. To extend these findings, recent work showed that ICV or intraperitoneal administration of insulin produces activation of the insulin receptor substrate (IRS)/phosphatidylinositol 3-kinase (PI3K) signaling pathway in the hypothalamus of rats [6,26] and that inhibition of PI3K with ICV wortmannin specifically blocked insulin-induced activation of lumbar SNA [6]. These results indicate that PI3K in the CNS may act as a specific mediator of the action of insulin on lumbar sympathoactivation. In the context of the present study, it is noteworthy that dietary-salt loading in rats causes increased insulin-induced activation of the IRS/PI3K pathway and increased phosphorylation of the downstream Akt pathway in liver and muscle tissues [27,28], whereas dietary salt depletion causes decreased insulin-induced activation of the IRS/PI3K/Akt pathway in the same tissues [29]. Although an effect of NaCl on PI3K/Akt in the CNS has not been investigated, we speculate that high NaCl causes increased insulin-induced activation of the IRS/PI3K/Akt pathway in the hypothalamus, which, in turn, produces increased lumbar sympathetic neural responses to insulin administration.

Insulin also evoked increases in HR that tended to parallel the sympathoactivation; however, the level of dietary NaCl did not affect the magnitude of the tachycardiac responses. Although this may appear to conflict with the SNA findings, it should be recalled that HR responses reflect not only changes in cardiac SNA, but also alterations in parasympathetic activity, direct effects on the sinus node, atrial reflexes, and responses to epinephrine, some of which may be influenced by NaCl. Indeed, previous work indicates that HR increases due to euglycemic hyperinsulinemia may be due, in part, to direct sinoatrial effects [30] and partly to parasympathetic withdrawal [31]. These interacting physiologic mechanisms may explain why dietary NaCl depletion in previous studies suppressed the gain of muscle SNA baroreceptor responses, but did not affect HR baroreceptor sensitivity [23-25], and why alterations in NaCl modified renal SNA responses to paraventricular stimulation and to thermal stimulation but did not significantly change the HR responses [19,20].

Although insulin caused increases in lumbar SNA in normal- and high-NaCl rats, it failed to produce elevations in MAP and actually resulted in mild blood pressure decreases across all 3 groups. Similar to this, Rahmouni and colleagues [6] recently demonstrated that acute intracerebroventricular administration of insulin produced simultaneous increases in lumbar, adrenal, renal, and brown adipose tissue SNA, but failed to elevate blood pressure in anesthetized rats. This lack of a pressor effect may be due to the presence of anesthesia and to the relatively short study duration, because chronic insulin administration in conscious rats consistently elevated blood pressure [6,11-13,17], whereas acute insulin in anesthetized animals alternately increased [18,32], did not change [2-6], or decreased [1,33] arterial pressure. In contrast, it may be that insulin produces sympathoactivation to metabolically active tissues, but not to the vasculature, to cause vasoconstriction. In support of a thermogenic or metabolic action of lumbar sympathoactivation, recent studies showed that hypothalamic blockade of insulin signal transduction not only prevented the weight-reducing effect of insulin administration, but also inhibited lumbar SNA increases in rats [6,26,34].

There were a number of limitations to the present study. First, we did not detect any differences in resting SNA between the 3 dietary groups. Because of the difficulty in comparing baseline nerve activities between animals due to nerve characteristics and electrode placement, it is possible that there were between-group differences in resting SNA that we failed to detect. In previous work, some groups reported higher basal SNA in rats given low dietary NaCl compared with medium or high NaCl [19,20,22]; however, others found no differences in baseline SNA in rats given a wide range of dietary NaCl [35-38]. A second limitation was that we recorded SNA only from the lumbar nerve, which sends sympathetic signals to muscle and skin vasculature in the hind limb of the rat. We therefore cannot extrapolate the present SNA findings to other vascular beds, and previous studies have shown that sympathoexcitatory stimuli may cause sympathetic changes in one vascular bed that may differ from changes in other sympathetic nerves. For instance, exogenous insulin administration has been shown to activate lumbar SNA but not renal or adrenal SNA in rats [3,5] and to activate muscle but not skin SNA in humans [7]. A final limitation was that these studies were carried out in urethane-anesthetized rats. Although all anesthetics are known to alter cardiovascular regulation, intravenous urethane has the special ability to induce a surgical plane of anesthesia without affecting neurotransmission in the peripheral nervous system or altering autonomic reflex responses [39,40].

In summary, 8 days of dietary NaCl loading in Sprague-Dawley rats exacerbated the increase in lumbar SNA due to acute euglycemic insulin infusion, whereas dietary NaCl

depletion attenuated the insulin-induced sympathoactivation. The strength of this modulation is reflected by 35% SNA increases in the low-NaCl group compared with 152% increases in the high-NaCl group (from a 100% baseline).

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