Impact of L-NAME on the cardiopulmonary reflex in cardiac hypertrophy

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Buckley MM, Johns EJ. Impact of L-NAME on the cardiopulmonary reflex in cardiac hypertrophy. Am J Physiol Regul Integr Comp Physiol 301: R1549–R1556, 2011. First published August 24, 2011; doi:10.1152/ajpregu.00307.2011.—There is evidence that in cardiac failure, there is defective baroreceptor reflex control of sympathetic nerve activity. Often, cardiac failure is preceded by a state of cardiac hypertrophy in which there may be enhanced performance of the heart. This study investigated whether in two different models of cardiac hypertrophy, there was an increased contribution of nitric oxide (NO) to the low-pressure baroreceptor regulation of renal sympathetic nerve activity (RSNA) and nerve-dependent excretory function. Administration of a volume load, 0.25% body wt/min saline for 30 min, in normal rats decreased RSNA by 40% and increased urine flow by some 9-fold. Following nitro-L-arginine methyl ester (L-NAME) administration, 10 μg·kg⁻¹·min⁻¹ for 60 min, which had no effect on blood pressure, heart rate, or RSNA, the volume load-induced renal sympathoinhibitory and excretory responses were markedly enhanced. In cardiac hypertrophy states induced by 2 wk of isoprenaline/caffeine or 1 wk thyroxine administration, the volume challenge failed to suppress RSNA, and there were blunted increases in urine flow in the innervated kidneys, but following L-NAME infusion, the volume load decreased RSNA by 30–40% and increased urine flow by some 20-fold in the innervated kidneys, roughly to the same extent as observed in normal rats. These findings suggest that the blunted renal sympathoinhibition and nerve-dependent diuresis to the volume load in cardiac hypertrophy are related to a heightened production or activity of NO within either the afferent or central arms of the reflex.

CARDIAC HYPERTROPHY, DEFINED as an increase in the size and/or thickness of the ventricles in the heart, frequently progresses into heart failure (2) and is an independent predictor of cardiovascular morbidity and mortality (5, 14). The development of cardiac hypertrophy is associated with an increased workload and an activation of the sympathetic nervous system, which drives the heart to ensure an adequate cardiac output and, if prolonged, may progress into cardiac failure. In the kidney, the raised sympathetic outflow will cause an enhanced sodium retention and raised circulatory volume, which will also contribute to the increased load on the heart (10).

The reflex control of the cardiovascular system and blood pressure depends not only on arterial baroreceptors, but also on receptors located in the cardiopulmonary region. It is generally recognized that increasing circulatory volume leads to a fall in renal sympathetic nerve activity (RSNA), and vice versa, and has been termed the cardiopulmonary reflex. Interestingly, in two models of cardiac hypertrophy induced by isoprenaline/caffeine or thyroxine exposure, it was found that the reflex renal sympathoinhibition in response to an acute saline volume load did not occur (4). This implied a deficit in the reflex residing either in the heart or within central pathways. These findings closely corresponded with observations in rat models of cardiac failure induced by coronary artery ligation (19) that the reflex regulation of renal sympathetic nerve activity by both the high- and low-pressure baroreceptors was blunted.

Despite the increasing evidence of important abnormalities in autonomic regulation of function in heart disease, the mechanisms underlying this deterioration remain poorly understood. A body of evidence has suggested that deficits in endogenous nitric oxide (NO) production may be involved. Studies by Zucker et al. (27) clearly observed that overexpression of nitric oxide synthase (NOS), and hence increased NO generation in an area of the brain associated with volume regulation, the paraventricular nucleus (PVN), restored the reflex regulation of RSNA. These findings demonstrated that in cardiac failure, there was a depletion of NO within at least one key area of the brain, which contributed to the deranged sympathetic regulation. Whether similar deficits exist in relation to the contribution of NO to cardiac function has received little attention. At the level of the heart, there is evidence that both endothelial NOS and neuronal NOS are expressed in the cardiac myocytes and that NO generation enhances contractility and its adrenergic regulation (18, 20, 26). There is also the possibility that NO may attenuate the sensory transduction mechanisms of cardiac afferent nerves determining the signaling to the central nervous system (18).

The question arises as to when this derangement in NO generation might take place, that is, does it precede the development of cardiac failure, when hypertrophy may be present, or is it a consequence of the reduced output from the failing heart. This investigation set out to explore the hypothesis that in states of cardiac hypertrophy, blockade of NO production would increase the degree of RSNA inhibition in response to an acute saline volume load. This was extended to determine the impact of the withdrawal of RSNA on renal excretory function. The approach taken was to evaluate RSNA and urine flow in response to a saline load before and following systemic administration of L-NAME in normal rats and those with cardiac hypertrophy induced by chronic administration of either isoprenaline/caffeine or thyroxine (4).

MATERIALS AND METHODS

Male Wistar rats, 190–240 g, were either bred in-house or purchased from commercial suppliers and maintained under a 12:12-h dark-light regime at 20 ± 3°C and 35% humidity. All procedures were performed in accordance with European Community Directive 86/609/EC and with the approval of the local Animal Experimentation Ethical Committee at University College Cork.

Vehicle rats were maintained on a regular diet and tap water but given subcutaneous injections of vehicle (saline, 0.1 ml/100 g body wt) every 24 or 72 h. To induce cardiac hypertrophy, groups of rats received either a normal diet and drinking water containing caffeine (62 mg/l) and were injected subcutaneously with isoprenaline (5

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mg/kg) every 72 h for 2 wk (4); or a normal diet and tap water and were injected subcutaneously with thyroxine (1 mg/kg) every 24 h for 1 wk (8).

Surgical procedure. All rats had their food restricted overnight prior to the study, but they were allowed free access to water or water plus caffeine. The last injection of the isoprenaline or thyroxine was at least 24 h prior to study. Anesthesia was induced with an intraperitoneal injection of a chloralose/urethane mixture (16.5 and 250 mg/ml, respectively) of 0.8–1 ml initially and maintained with supplemental doses of 0.05 ml iv every 30 min. A cannula was inserted into the trachea, and the animal was allowed to breathe spontaneously. Cannulas were placed in the femoral vein for saline infusion (150 mM NaCl; 3 ml/h) and drug administration, and in the femoral artery for monitoring of blood pressure and heart rate. A cather was inserted into the bladder to allow urine to drain. The left kidney was exposed retroperitoneally by a left flank incision. The renal sympathetic nerves were isolated, cleared of connective tissue, and a renal nerve bundle was placed on stainless-steel recording electrodes and sealed in place with dental glue.

Blood pressure was monitored using a pressure transducer (Spectramed, Oxnard, CA) and an amplifier (Grayden Electrornics, Birmingham, UK). RSNA was amplified with a gain of 100,000 and high- and low-pass filters set at 0.2 and 2 kHz. The blood pressure and renal sympathetic nerve signals were distributed into an audio amplifier and onto the monitor screen of the computer. The signals were digitized to enable generation of mean blood pressure and integrated RSNA, and they were stored onto the hard disk every 1 s and used in later off-line analysis.

RSNA study. The animals were allowed to stabilize after the surgical procedure for a period of 1½ to 2 h, after which a 5-min baseline recording was taken of mean arterial pressure (MAP), heart rate (HR), and RSNA. A control acute saline volume expansion was carried out at 0.25% body weight/min for 30 min, followed by a 30-min recovery period. l-NAME was then administered intravenously at a rate of 10 μg·min⁻¹·kg⁻¹ for 60 min. Thereafter, a second volume expansion protocol was performed. At the end of the protocol, the animals were killed by an overdose of anesthetic given intravenously.

Renal function study. The initial surgical procedure was as described above. The right kidney and ureter were exposed by a right flank incision, and a cannula was inserted into the ureter and tied in place to permit urine collection. Following a left flank incision, the left kidney was exposed retroperitoneally, and the renal nerves were sectioned. The left ureter was isolated and cannulated for subsequent urine collection. FITC inulin 2 mg/ml in 0.9% NaCl was administered as a bolus dose of 2 ml immediately following surgery, and it was subsequently administered as an infusion at 1 ml/100 g body wt/h for the remainder of the experiment.

The animals were allowed to stabilize for a period of 1 h. Thereafter, 5-min baseline recordings of MAP and HR were taken. Blood samples were collected before and after two 15-min baseline clearances for measurement of inulin and electrolyte concentrations, to allow the calculation of glomerular filtration rate (GFR) and electrolyte excretion (9). Further, 5-min urine collections were taken during the 30-min volume expansion period, after which, another blood sample was taken. Thirty minutes later, two further 15-min clearance periods were taken for the recovery values. Thereafter, the full protocol was repeated, with either vehicle or l-NAME being infused. Cumulative urine flow was calculated as follows: once the rate of saline infusion was increased, the urine flow obtained during the first 5-min collection was added to the second period and so forth for the whole 30-min period, and the total volume obtained was termed “cumulative urine flow”.

l-NAME was infused at a rate of 10 μg·min⁻¹·kg⁻¹ for 60 min followed by a 15-min clearance. Thereafter, a second 30-min volume expansion was performed. At the end of the experiment, the animals were killed by an overdose of anesthetic given intravenously.

Experimental groups. Twelve groups of rats were used: four receiving vehicle, four receiving isoprenaline/caffeine, and four receiving thyroxine treatment.

Within each of the four groups, two groups were prepared for renal sympathetic nerve activity recording, one of which comprised two sequential saline volume expansion periods as time control experiments, the other where l-NAME was infused during the second volume expansion period.

The other two groups of rats were prepared for renal functional measurements; again, one group acted as a time control over the two volume expansion periods, while in the other group, after the first volume expansion, l-NAME was infused, and the second volume expansion protocol was performed.

Statistics. Data are presented as means ± SE. All values between and within groups were compared using a two-way repeated-measures ANOVA followed by Holm-Sidak post hoc analysis for specific means comparisons with reference to baseline values. Significance was taken when P < 0.05.

RESULTS

RSNA study. It is evident from Table 1 that the baseline levels of blood pressure, HR, and RSNA were very similar in the vehicle, isoprenaline/caffeine-treated, and thyroxine-treated rats. Table 2 shows that the infusion of l-NAME had no effect on blood pressure, heart rate, or renal sympathetic nerve activity in either the vehicle rats or those given either isoprenaline/caffeine or thyroxine.

The effect of the isoprenaline/caffeine and thyroxine treatment on body weight gain, heart weight, and heart weight-to-body weight ratio is shown in Table 3. It can be seen that the gain in weight over the treatment period was significantly (P < 0.05) less in both the isoprenaline/caffeine- and the thyroxine-treated groups than the group maintained on a regular diet. Table 3 also shows that both heart weights and heart weight-to-body weight ratios were significantly (P < 0.01 to 0.001) higher in the isoprenaline/caffeine- and thyroxine-treated compared with the rats given vehicle. Figure 1 illustrates the RSNA responses in groups of vehicle treated and heart hypertrophic rats subjected to two consecutive periods of acute volume expansion. It can be seen in the

Table 1. Mean arterial pressure, heart rate, and renal sympathetic nerve activity at baseline and following 30-min volume expansion in rats treated with vehicle, isoprenaline/caffeine, or thyroxine

<table>
<thead>
<tr>
<th></th>
<th>Vehicle (n = 6)</th>
<th>Isoprenaline/Caffeine (n = 6)</th>
<th>Thyrroxine (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>86 ± 4</td>
<td>97 ± 4</td>
<td>96 ± 3</td>
</tr>
<tr>
<td>Heart rate, Hz</td>
<td>6.7 ± 0.2</td>
<td>6.5 ± 0.3</td>
<td>6.6 ± 0.4</td>
</tr>
<tr>
<td>Renal sympathetic nerve activity, μV</td>
<td>8.5 ± 0.8</td>
<td>4.3 ± 0.3</td>
<td>6.4 ± 0.2</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. **P < 0.01 compared with baseline.
The first period of acute saline volume expansion did not significantly (P > 0.01) decrease in RSNA reaching some 44% by the end of the first infusion period, which had recovered close to baseline levels 30 min later. The second period of acute saline volume expansion also resulted in an approximate 45% (P < 0.01) reduction in RSNA, which also recovered close to baseline values in the recovery period. The magnitudes of the renal sympathoinhibition achieved in the two periods of saline volume expansion were identical. Figure 1B compares the two sequential acute saline volume expansion challenges in the isoprenaline/caffeine-treated rats, and it is evident that neither the first nor the second saline volume challenge altered the level of RSNA. The acute saline volume expansion in the thyroxine-treated rats (Fig. 1C) did not change renal sympathetic nerve activity in either the first or second challenges. The lack of change in RSNA in response to the acute saline volume expansion in the isoprenaline/caffeine and thyroxine groups of rats was significantly different from the reduction in RSNA observed in the vehicle-treated rats (both P < 0.01).

The effect of L-NAME administration on the renal sympathetic nerve responses to an acute saline volume expansion is given in Fig. 2. The vehicle-treated rats (Fig. 2A) reversibly decreased RSNA by 45% (P < 0.01) before and 78% (P < 0.01) following the L-NAME infusion in response to the two periods of acute saline volume expansion, with the latter being significantly (P < 0.01) larger than the former. It was evident in the isoprenaline/caffeine-treated rats (Fig. 2B) that although the first period of acute saline volume expansion did not change RSNA, following the infusion of L-NAME, the acute saline volume expansion produced a significant (P < 0.01) decrease of some 33%. Similarly, in the thyroxine-treated rats (Fig. 2C), the first volume challenge was without effect on RSNA, but after the infusion of L-NAME, the volume expansion significantly (P < 0.01) reduced RSNA by some 40%. The magnitudes of the renal sympathoinhibition in response to the second volume expansion were significantly larger (all P < 0.01) in all groups of rats in the presence of L-NAME than in its absence.

Renal functional study. The baseline values for glomerular filtration rate were similar for both kidneys in all three groups of rats (Table 4), while urine flow and absolute and fractional sodium excretions in the left denervated kidneys were significantly higher than in the right innervated kidneys. Table 4 also provides the changes in renal excretory data following the L-NAME infusion. There were no significant changes in GFR or absolute or fractional sodium excretions in either the left innervated or right denervated kidneys following the L-NAME infusion, although urine flow was increased (P < 0.01) in the innervated kidneys of the vehicle rats. A similar pattern of response to L-NAME was also observed in the isoprenaline/caffeine- and thyroxine-treated groups.

The saline volume expansion in the control group of vehicle rats (Fig. 3A) resulted in a greater increase in the cumulative urine flow from the denervated than innervated kidneys in both the first and second (by some 60 to 70%, P < 0.01) periods of acute saline volume expansion, and the magnitude of these responses was not different from each other. In the isoprenaline/caffeine (Fig. 3B), the cumulative urine flow in response to the acute saline volume expansion was markedly reduced in the innervated kidney compared with the denervated kidney in both the first and second challenges, but the magnitude of the responses was not different in the first and second volume expansion periods. The cumulative urine flow response to the two consecutive acute saline volume expansion challenges in the thyroxine-treated group is shown in Fig. 3C. It is apparent that the cumulative urine flow was markedly reduced in the innervated kidneys compared with the denervated kidneys in both the first and second acute saline volume challenges. The magnitude of the responses obtained from the two kidneys was identical in the first and second acute saline volume periods, and the magnitudes in the denervated kidneys were comparable to the value obtained in the innervated kidneys of the vehicle rats (Fig. 3A).

Figure 4 presents the data obtained where, after the first acute saline volume expansion, L-NAME was infused, and a second volume challenge was performed in its presence. In the vehicle-treated rats (Fig. 4A), there was a significantly (P < 0.01) larger increase in urine flow from the denervated than the innervated kidney. In the presence of L-NAME, the cumulative urine flow was significantly (P < 0.01) greater in both kidneys than that obtained in its absence. It was evident (Fig. 4B) in the isoprenaline/caffeine group of rats that the acute volume expansion caused small insignificant changes in cumulative urine flow in the innervated kidney and a significantly (P < 0.01) greater increase in the denervated kidney. The responses in

Table 3. Comparison of heart and body weights of normal vehicle-treated rats with those treated with isoprenaline and caffeine for 2 wk or thyroxine for 1 wk

<table>
<thead>
<tr>
<th></th>
<th>Vehicle (n = 6)</th>
<th>Isoprenaline/Caffeine (n = 6)</th>
<th>Thyroxine (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before treatment</td>
<td>205 ± 6</td>
<td>206 ± 3</td>
<td>203 ± 5</td>
</tr>
<tr>
<td>Following treatment</td>
<td>234 ± 3</td>
<td>214 ± 7</td>
<td>215 ± 4</td>
</tr>
<tr>
<td>Weight gained, g</td>
<td>28 ± 4</td>
<td>8 ± 2**</td>
<td>11 ± 7*</td>
</tr>
<tr>
<td>Heart weight, mg</td>
<td>627 ± 28</td>
<td>834 ± 40**</td>
<td>712 ± 51**</td>
</tr>
<tr>
<td>Heart weight/body weight ratio, 10⁻³</td>
<td>2.68 ± 0.13</td>
<td>3.89 ± 0.01***</td>
<td>3.31 ± 0.11***</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01, ***P < 0.001 vehicle compared with isoprenaline/caffeine or thyroxine treatment.
cumulative urine flow were significantly \((P < 0.01)\) larger in both kidneys in the presence of L-NAME than in its absence. In the thyroxine model of cardiac hypertrophy, the first volume expansion caused a much larger increase in cumulative urine flow in the denervated compared with the innervated kidney \((P < 0.01)\). The magnitudes of the cumulative urine flow responses to the volume expansion from both kidneys were significantly \((both P < 0.01)\) enhanced in the presence of L-NAME.

DISCUSSION

There is clinical and experimental evidence that in cardiac failure (3, 12, 17), the normal suppression of sympathetic outflow to an acute saline volume load is lost and, in relation to RSNA, appears to be due to a reduction in NO production, leading to an exaggerated baroreflex control (13, 23). The primary objective of the study, herein, was to investigate the contribution of NO to the renal sympathoinhibition and the renal nerve-dependent diuretic responses to an acute volume expansion and how these relationships were modified in states of cardiac failure (3, 12, 17).

Fig. 1. The renal sympathetic nerve activity responses to two sequential acute saline volume expansions in groups of rats treated with vehicle \((A)\), isoprenaline/caffeine \((B)\), or thyroxine \((C)\). \(*P < 0.01\), compared with baseline. \$\(P < 0.01\), compared with vehicle-treated rats.

Fig. 2. The renal sympathetic nerve activity responses to acute saline volume expansion before and following nitro-L-arginine methyl ester (L-NAME) infusion in groups of rats treated with vehicle \((A)\), isoprenaline/caffeine \((B)\), or thyroxine \((C)\). \(*P < 0.01\), compared with baseline. \#\(P < 0.001\), compared with baseline. \$\(P < 0.01\), compared with vehicle-treated rats.
hypertrophy and before cardiac function was impaired. The experimental design was such that each animal was subjected to two periods of saline volume expansion. It was clear that the magnitudes of the RSNA responses and the renal excretory responses were very reproducible over the time frame of the experiment. The studies revealed that infusion of L-NAME actually enhanced not only the magnitude of the renal sympathoinhibition but also the renal nerve-dependent excretory response to a saline volume load. In the cardiac hypertrophy models, the renal sympathoinhibition and neural component of the diuresis resulting from a saline volume load was not present, but it was restored following blockade of NO production. These findings support the hypothesis that in cardiac hypertrophy, but before there is a

Table 4. Comparisons of glomerular filtration rate, urine flow, absolute sodium excretion, and fractional sodium excretion in the innervated and denervated kidneys of rats treated with vehicle, isoprenaline/caffeine, or thyroxine

<table>
<thead>
<tr>
<th></th>
<th>Innervated (n = 8)</th>
<th>Denervated (n = 8)</th>
<th>Innervated (n = 7)</th>
<th>Denervated (n = 7)</th>
<th>Thyroxine (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>t-NAME</td>
<td>Baseline</td>
<td>t-NAME</td>
<td>Baseline</td>
</tr>
<tr>
<td>GFR, ml·min⁻¹·kg⁻¹</td>
<td>2.3 ± 0.3</td>
<td>2.4 ± 0.3</td>
<td>2.5 ± 0.3</td>
<td>2.5 ± 0.4</td>
<td>1.9 ± 0.1</td>
</tr>
<tr>
<td>UF, µl·min⁻¹·kg⁻¹</td>
<td>22.8 ± 4</td>
<td>46.2 ± 4.3#</td>
<td>50.7 ± 9*</td>
<td>58.2 ± 5.5</td>
<td>6.4 ± 0.4</td>
</tr>
<tr>
<td>UNaE, µmol·min⁻¹·kg⁻¹</td>
<td>0.4 ± 0.1</td>
<td>0.18 ± 0.1</td>
<td>1.3 ± 0.3*</td>
<td>1.33 ± 0.2</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>FNaE, %</td>
<td>1.5 ± 0.1</td>
<td>1.4 ± 0.3</td>
<td>2.1 ± 0.3*</td>
<td>2.2 ± 0.2</td>
<td>1.9 ± 0.2</td>
</tr>
</tbody>
</table>

GFR, glomerular filtration rate; UF, urine flow; UNaE, absolute sodium excretion; FNaE, fractional sodium excretion. *P < 0.01, denervated compared to innervated #P < 0.01. L-NAME vs. baseline.

Fig. 3. Cumulative urine volume following two sequential acute saline volume expansion periods in rats treated with vehicle (A), isoprenaline/caffeine (B), or thyroxine (C). *P < 0.01, compared with innervated kidney, denervated compared to innervated.
deterioration in cardiac function, the loss of the reflex renal sympathoinhibition and renal nerve-dependent diuresis to the volume expansion was related to an enhanced tonic inhibition due to an elevated contribution of NO.

The saline volume load in the normal rats would have increased central venous volume, activating the cardiopulmonary receptors and eliciting a decrease in sympathetic outflow (4), as well as causing the release of atrial natriuretic peptide. Interestingly, although there was a marked reduction in RSNA as a result of the saline load, there was no change in blood pressure or heart rate, suggesting that there was little withdrawal of sympathetic activity from other regions. Indeed, there is support for the view that alterations in vascular volume preferentially determines sympathetic outflow to the kidney (16). This reduction in RSNA caused by the saline load had renal excretory consequences in that the increase in urine flow was greater in the denervated compared with the innervated kidney, which would be consistent with the renal sympathetic nerves not being totally suppressed but still able to exert a fluid-retaining action.

L-NAME was given as a slow infusion at 10 μg·min⁻¹·kg⁻¹ over a 60-min period with a view to blocking NOS. Indeed, acute intravenous administration of a comparable dose of L-NAME by Grzelec-Mojzesowicz and Sadowski (7) caused an almost maximal suppression of NO concentration in the kidney, and a similar situation is likely to pertain in other tissues, such as the heart and brain, as L-NAME is able to cross the blood-brain barrier (22). Administration of L-NAME had no effect on blood pressure or heart rate, although RSNA was slightly decreased. These observations concur with those of Gentilcore et al. (6) and Lahera et al. (11), who used similar rates of infusion that did not alter blood pressure or heart rate for the period of the infusion. There was little impact on either glomerular filtration rate or sodium excretion, which was very similar to that reported by Lahera et al. (11). Although there was a consistent increase in urine flow from the innervated kidneys, the underlying reasons are unclear. The first novel finding was that blockade of NOS with L-NAME caused an enhanced renal sympathoinhibition to the saline load, which was also reflected at the functional level, insofar as the diuretic response was also greater consistent with the larger withdrawal of sympathetic tone. The question arises as to which part of the reflex pathway, afferent, central, or efferent arm was modified by the L-NAME given systemically in this way. Clearly, there was a close correlation between the nerve activity and renal nerve-dependent excretory responses, suggesting that at the level of the kidney, the effect of L-NAME was limited. This would indicate that either the afferent or central components are more involved. In terms of the central nervous system, systemic administration of NOS blockers causes a sympathoexcitation (21), while application of NO donors onto the nucleus of the solitary tract depresses activity of neurons in the PVN (1), and at the PVN, NO donation decreased and L-NAME increased renal sympathetic nerve activity (24). At the level of the heart, NO may be acting at two levels, within the cardiac myocytes and at the sensory transduction mechanisms. It has been reported (18, 20, 26) that NO supports and elevates contractility of the heart. This would argue that in the present study L-NAME might decrease contractility, and for output to be maintained, there could be a reflex increase in cardiac sympathetic activity, as well as outflow to other organs such as the kidney. The contribution of NO in signaling transduction at the level of the cardiopulmonary receptors is unclear, but there is evidence that at vagal afferent nerves in the gut, L-NAME caused marked increases in responsiveness to sensory stimuli (18). In terms of the present study, attenuation of NO generation at the cardiopulmonary receptors would increase their sensitivity, resulting in an enhancement of the reflex sympathoinhibition observed at the kidney.

Two models of cardiac hypertrophy were used, one involving 2-wk administration of isoprenaline/caffeine and the other...
involving 1-wk administration of thyroxine, which has been previously shown to cause a cardiac hypertrophy associated with a blunted renal sympathoinhibitory response to a saline load (4). Importantly, the cardiac hypertrophy enhanced function as cardiac contractility was increased in the isoprenaline/caffeine model (4). The present study has provided further novel insights, in that in the two models of cardiac hypertrophy, the blunted renal sympathoinhibition to the saline load was translated into a functional consequence as the excretory response to the saline load was markedly suppressed in the innervated kidneys, but not in the kidneys subjected to the acute denervation, where they were comparable to those obtained in intact kidneys of normal rats.

Administration of l-NAME at the dose used had minimal effects on blood pressure, heart rate, glomerular filtration rate, and basal levels of sodium excretion in innervated and denervated kidneys in both models of cardiac hypertrophy. However, in both models, there was an increase in urine flow in the innervated but not denervated kidneys following infusion of the l-NAME. As noted above, the underlying causes of this diuretic action are uncertain.

The third novel finding was that in both the isoprenaline/caffeine and the thyroxine models of cardiac hypertrophy following the l-NAME infusion, RSNA was decreased by some 40% during the saline load, almost equivalent to that obtained in the normal rats. Moreover, in the presence of l-NAME, the excretory responses were greatly enhanced in the innervated kidneys compared with those obtained when l-NAME was not given. Thus, together, the changes in renal sympathetic nerve activity and the neurally dependent fluid excretory responses appeared to be compatible with excessive inhibitory control of NO in the cardiac hypertrophy states. The site at which NO may be exerting this inhibitory control, within the heart or at the central nervous system cannot be ascertained using the current study design of systemic administration of l-NAME but does point the way to future studies.

Perspectives and Significance

The present report on the regulation of renal sympathetic nerve activity and nerve-dependent excretory function clearly shows that in the two models of cardiac hypertrophy there is a heightened tonic inhibitory role for NO. This situation contrasts with that in rat models of cardiac failure induced by coronary artery ligation, where there appears to be a depletion of NO production or activity, at least in the autonomic control nuclei in the brain. It is important to point out that in these cardiac failure models, studies were performed 4–6 wk after surgery, which contrasts with the 1- to 2-wk time frame of the cardiac hypertrophy studies reported herein, and it may be that the involvement of NO is increased in the initial phase but becomes depressed as the work load on the remaining functional cardiac tissue becomes prolonged. On the other hand, both models used in the current study employ an elevated adrenergic drive to the heart to induce hypertrophy, which parallels that taking place during exercise training in man. Indeed, there are reports in rats exposed to exercise training that the contribution of NO to tonically suppress sympathetic outflow is enhanced (15, 25), although it was not ascertained whether these levels of exercise resulted in an increase in heart weight. Together, previous reports and the current findings point to a significant relationship between the work required by the heart and the level of NOS and NO activity in the reflex regulation of sympathetic outflow, particularly to the kidney. It is unclear at present whether the deficit lies in afferent or central components of the reflex.

REFERENCES


