Interactions between nitric oxide and renal nerves in the excretion of a saline load in obese Zucker rats

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Abstract
The present study investigated the potential role of nitric oxide (NO) and its interaction with renal sympathetic nerves in modulating the excretory responses to an acute saline volume expansion (VE), of 10% of body weight, in the innervated and denervated kidneys of both lean and obese Zucker rats. This was done using the NO synthase inhibitors \( \text{N}^\text{G}-\text{nitro-L-arginine methyl ester} \) (L-NAME), 7-nitroindazole and aminoguanidine. In lean rats, cumulative urinary sodium excretion (\( \text{cu}U_{\text{Na}}V \)) after 40 min of VE in the innervated kidney was enhanced by 48% in L-NAME-treated rats compared with that in untreated rats, but this was not the case for the denervated kidney. VE in untreated obese rats raised \( \text{cu}U_{\text{Na}}V \) to a lesser extent than in the untreated lean rats, by 36% and 46% in the denervated and innervated kidneys respectively (both \( P < 0.001 \)). L-NAME treatment of obese rats increased \( \text{cu}U_{\text{Na}}V \) after VE compared with that in untreated obese rats, by 48% in the denervated kidney and by 136% in the innervated kidney (both \( P < 0.001 \)). The magnitude of \( \text{cu}U_{\text{Na}}V \) after VE in both kidneys of 7-nitroindazole-treated obese rats was not different from that in untreated obese rats. However, \( \text{cu}U_{\text{Na}}V \) was raised (\( P < 0.01 \)) by 56% in the innervated, but not the denervated, kidney of aminoguanidine-treated obese rats. These data show that NO is partially involved in mediating the reflex renal responses to VE in Zucker rat strains. NO, possibly generated by endothelial NO synthase, exerts its effects in obese rats through a renal-nerve-independent mechanism, while the effect of NO generated by inducible NO synthase requires intact renal innervation.

Introduction
Obesity is increasingly accepted as being a major cause of human essential hypertension [1,2], but the pathophysiological mechanisms underlying the weight-related increases in blood pressure remain undetermined. A prevailing concept is that sodium retention and expansion of the extracellular fluid volume are consistent features of obesity, and the lack of physiological adaptation to these changes may contribute to the development of obesity-related hypertension [1–4]. An abnormal pressure–natriuresis relationship [3–7], as well as an attenuated natriuretic response to an acute sodium load [8–10], have been reported in fat-fed obese dogs and genetically obese Zucker rats. The mechanisms underlying these abnormalities are still a matter of debate, but Zeigler and Patel

Key words: kidney function, nitric oxide, obesity, sodium excretion, volume expansion.
Abbreviations: FE_{\text{Na}}, fractional sodium excretion; GFR, glomerular filtration rate; MAP, mean arterial pressure; \text{-NAME}, \text{N}^\text{G}-\text{nitro-L-arginine methyl ester}; NOS, nitric oxide synthase; eNOS, nNOS and iNOS, endothelial, neuronal and inducible isoforms of NOS respectively; RBF, renal blood flow; (cu)UV, (cumulative) urine flow; (cu)U_{\text{Na}}V, (cumulative) absolute urinary sodium excretion; VE, volume expansion.
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[9] have reported that the volume reflex that defends the body against changes in extracellular fluid volume is impaired in obesity.

A possible role for enhanced sympathetic nervous system activity in obesity-associated hypertension has been documented [1,11–14]. Basal plasma noradrenaline levels and the noradrenaline responses to stimuli such as upright posture and isometric handgrip have been reported to be elevated in obese compared with lean subjects [1,8,15]. Acute ganglionic or adrenergic blockade caused a greater reduction in blood pressure in fat-fed obese dogs compared with lean dogs [16], while renal denervation markedly attenuated sodium retention and hypertension associated with obesity [8]. Furthermore, experiments in which renal sympathetic nerve activity was recorded have shown elevated basal efferent renal nerve activity in both genetic and fat-fed obese rats [11,13,14]. These pieces of evidence give rise to the suggestion that the reflex-mediated responses in sympathetic nerve activity to the kidney may be altered in obesity, and that sodium retention, as a consequence of the direct action of the sympathetic nerves on tubular sodium reabsorption, might lead indirectly to an increase in blood pressure.

A number of humoral as well as paracrine/autocrine factors are now recognized as modulating the impact of the renal nerves on their functional end points in the kidney. One of these is nitric oxide (NO), which is the oxidative product of L-arginine produced by the enzyme NO synthase (NOS). NOS exists in three isoforms: endothelial (eNOS), neuronal (nNOS) and inducible (iNOS). It is apparent that NOS is widely distributed in the kidney, with eNOS being present along the vasculature and diffusely along the nephrons, whereas nNOS is localized at high concentrations at the macula densa and at a lower concentration at the inner medullary collecting duct [17–19]. In terms of functionality, NO can influence renal fluid reabsorption and appears to act in an inhibitory fashion on epithelial cell transport [18,20–23]. Finally, a number of reports have highlighted the fact that NO can exert a modulatory role on neurotransmission, including that by sympathetic nerves, which may also come into play at the level of the kidney [24–28]. There is now a body of evidence demonstrating elevated generation of NO in obesity, both in vivo and in vitro [29–33]. This means that the neural regulation of fluid handling by the kidney may well be deranged in obesity and contribute, in part, to the associated hypertension.

Studies were undertaken in the Zucker obese and lean rat strains to evaluate the contribution of the renal nerves and NO to the deranged volume reflex in obesity. This was done by comparing the renal excretory responses in innervated and denervated kidneys with intact NO synthesis and following selective blockade of NOS isoforms.

METHODS

All experiments were carried out under the auspices of the U.K. Government project licence PIL 40/1367, and personal investigator licences PIL 40/00371 (E.J.J.) and PIL 40/5301 (O.W.). Normal ethical requirements for animal research were complied with at all times.

Animal preparation

Experiments were conducted in male Zucker obese and lean rats (Harlan, Bicester, Oxon., U.K.) aged 12–14 weeks, which has been reported to be the age of peak insulin levels [9]. The animals were maintained in the Animal Facility on a 12 h/12 h light/dark cycle at an ambient temperature of 18–22 °C and a humidity of 45–55%. Rat pellets (SDS Breeding Diet) were supplied ad libitum. The animals had their food restricted overnight before the experiments, but were allowed free access to water.

Anaesthesia was induced with a mixture of fluothane/O2/N2O, and was maintained throughout the course of the experiment by intravenous boluses of a chloralose/urethane mixture (12 and 180 mg ml−1 respectively) of 0.7–0.9 ml initially and then 0.05 ml every 30 min or as required. A tracheal cannula was inserted and the animal breathed spontaneously. Cannulae were placed in the right femoral vein for saline infusion (150 mM NaCl; 3 ml h−1) and the right femoral artery for monitoring of mean arterial pressure (MAP) and blood sampling. Through flank incisions, both the right and left ureters were cannulated for urine collection, and the left kidney was denervated using a dissecting microscope by stripping off all visible nerve fibres penetrating the renal hilus. Renal denervation was considered complete when the renal vasoconstrictor response to direct electrical stimulation (15 V, 10 Hz, 0.2 ms for 10 s) of the proximal renal nerve bundle was abolished. An electromagnetic flow probe (EP100 series; internal circumference 2.5 mm) was fitted around the left renal artery and connected to a square-wave flowmeter (FM 501; Carolina Medical Electronics, Inc., King, NC, U.S.A.) to measure renal blood flow (RBF). Following completion of surgery, a priming dose of 2 ml of insulin in saline (1.5 g·100 ml−1) was given intravenously, followed by a sustained infusion of 3 ml h−1. The animals were allowed to stabilize for 2 h before starting the experimental protocols.

Experimental protocols

Protocol 1: renal responses to volume expansion (VE)

This study was carried out to evaluate the renal responses to acute VE in obese (n = 7) and lean (n = 9) Zucker rats. After the animal had been stabilized, two 15 min baseline urine collections were obtained, and then VE was performed by intravenous infusion of isotonic saline at 0.25% of body weight min−1 for 40 min (total VE of
10% of body weight), with 5 min clearances being taken during this period. Arterial blood samples were collected at the beginning of the experiment, at the end of the baseline period and at the end of VE for determination of plasma inulin and sodium. Blood glucose was tested only once, in the first blood sample. At the end of the experiment, the animals were killed by a bolus injection of pentobarbital sodium (60 mg intravenous), and both kidneys were removed, decapsulated, blotted dry and weighed in order to standardize renal function measurements on a kidney weight basis.

Protocol 2: effects of L-NAME on renal haemodynamics and excretory functions

This series of experiments explored the effects of blocking endogenous NO production on renal haemodynamic and excretory function both at baseline and during the VE period in obese (n = 10) and lean (n = 11) Zucker rats. After baseline clearances were taken, Nω-nitro-L-arginine methyl ester (L-NAME; a non-selective NOS inhibitor) was given intravenously at a rate of 10 μg min⁻¹ kg⁻¹ until the end of the experiment. The dose of L-NAME used was based on previous investigations which showed that it was effective at blocking NOS in the rat kidney [20]. An interval of 60 min was allowed in order for L-NAME to equilibrate and develop its full effect before two further 15 min clearances were taken to obtain new baseline values; VE was then started and all clearance collections were carried out as outlined in protocol 1.

Protocol 3: characterization of specific NOS isoform

To characterize which isoform(s) might be responsible for the effects seen after blocking NO production with L-NAME in obese Zucker rats, two additional subgroups of obese Zucker rats (n = 9–10) were treated with either 7-nitroindazole (a relatively selective blocker of nNOS) or aminoguanidine (a relatively selective blocker of iNOS). The compound was given after collection of the first two baseline clearances; 30 min later, the animals underwent a similar experimental protocol as described in protocol 2. A single bolus dose of 7-nitroindazole at 50 mg kg⁻¹ was dissolved in 0.3–0.4 ml of arachis oil and injected intraperitoneally; this dose produces substantial inhibition of nNOS within 30 min without altering MAP or the vasodilator response to acetylcholine [34]. Aminoguanidine was dissolved in saline and delivered intravenously at 1.666 mg min⁻¹ kg⁻¹ for the duration of the experiment. This dose of aminoguanidine has been shown to significantly decrease plasma nitrate and nitrite levels without eliciting a pressor response [35].

Chemicals

7-Nitroindazole was obtained from RBI (Natick, MA, U.S.A.), and arachis oil was from Hillcross Pharmaceuticals (Burnley, U.K.). All other compounds were purchased from Sigma (St. Louis, MO, U.S.A.).

Analytical methods

The blood glucose concentration was measured using a glucometer (model 5529; Miles Laboratories Inc., Elkhart, IN, U.S.A.). Urine volume was measured gravimetrically. The sodium concentrations in urine and plasma samples were analysed by flame photometry (model 410 C; Ciba Corning, Halstead, Essex, U.K.). Plasma and urine inulin concentrations were determined using a diphenylamine colorimetric technique [36], and the glomerular filtration rate (GFR) was calculated based on the renal clearance of inulin.

Statistical analysis

Data are expressed as means ± S.E.M. Differences within groups were tested using the Student’s paired t test. Comparison of means among groups was analysed using one-way ANOVA followed by a Bonferroni/Dunn post-hoc test. Repeated-measures ANOVA was used to compare the profiles of the responses during the VE period among the groups. All statistical calculations were performed with SuperANOVA and Statview software (Abacus Concepts, Berkeley, CA, U.S.A.), and significance was taken at P < 0.05.

RESULTS

Protocol 1: renal responses to VE

The obese Zucker rats were significantly heavier than their lean age-matched littermate controls (512 ± 7 g and 292 ± 12 g respectively; P < 0.001) and had significantly higher levels of plasma glucose (7.2 ± 0.5 and 4.8 ± 0.1 mmol l⁻¹ respectively; P < 0.001). Although the weights of both the left and right kidneys of the obese animals were significantly greater than those recorded in the lean animals (1.36 ± 0.05 and 0.98 ± 0.0 g respectively for the left kidney, and 1.57 ± 0.03 and 1.00 ± 0.04 g respectively for the right kidney; both P < 0.001), there was no difference in the kidney weight/body weight ratio between the groups. Basal MAP was approx. 10% higher in the obese compared with the lean rats (P < 0.01), and this was maintained throughout the remainder of the experiment, while heart rate and RBF were comparable at all time points (Table 1). Basal GFR was similar in both kidneys and between the groups (Table 1). However, basal urine flow (UV), absolute urinary sodium excretion (UnaV) and fractional sodium excretion (FenaNa) were all significantly higher in the denervated than in the contralateral innervated kidneys in both groups (all P < 0.01–0.05), while the basal excretion rates were greater in the lean than in the obese rats for both kidneys (Table 1).
O. Wongmekiat and E. J. Johns

Obese Zucker rats

Significance obtained by summing the absolute values for each collection period from the start to the finish of the VE period, and then subtracting the baseline value. Significance was calculated by adding up the absolute values at each time point from the beginning to the end of VE, and then subtracting the baseline value from this total.

The cumulative values (in units of l/min/gkwt) for GFR (ml/min/gkwt), RBF (ml/min/gkwt), and FE\textsubscript{Na} (%) were calculated by summing the absolute values at each time point from the beginning to the end of VE, and then subtracting the baseline value from this total.

Table 1. Effects of L-NAME on basalsystemic and renal haemodynamics and excretory functions in lean and obese Zucker rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Kidney</th>
<th>Lean Zucker rats</th>
<th>Obese Zucker rats</th>
<th>Before infusion</th>
<th>After infusion</th>
<th>Before infusion</th>
<th>After infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td>105 ± 1</td>
<td>109 ± 1**</td>
<td>112 ± 1†</td>
<td>110 ± 1*</td>
</tr>
<tr>
<td>Heart rate (beats·min(^{-1}))</td>
<td></td>
<td></td>
<td></td>
<td>397 ± 9</td>
<td>373 ± 8**</td>
<td>382 ± 7</td>
<td>362 ± 8**</td>
</tr>
<tr>
<td>RBF (ml·min(^{-1})·gkwt(^{-1}))</td>
<td>Denervated</td>
<td>6.9 ± 0.8</td>
<td>7.0 ± 0.7</td>
<td>7.2 ± 0.6</td>
<td>6.2 ± 0.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Innervated</td>
<td>1.1 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GFR (ml·min(^{-1})·gkwt(^{-1}))</td>
<td></td>
<td></td>
<td></td>
<td>1.0 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UV (l·min(^{-1})·gkwt(^{-1}))</td>
<td>Denervated</td>
<td>23.3 ± 3.0</td>
<td>30.2 ± 3.7</td>
<td>12.9 ± 2.9†</td>
<td>16.5 ± 3.0*</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Innervated</td>
<td>11.0 ± 1.5†</td>
<td>14.4 ± 1.7†</td>
<td>5.3 ± 1.1†</td>
<td>8.8 ± 1.1††</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(U_{Na})(V) (µmol·min(^{-1})·gkwt(^{-1}))</td>
<td>Denervated</td>
<td>3.6 ± 0.4</td>
<td>4.6 ± 0.4</td>
<td>2.0 ± 0.4†</td>
<td>2.4 ± 0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Innervated</td>
<td>1.7 ± 0.3†</td>
<td>2.0 ± 0.2†</td>
<td>0.5 ± 0.1†</td>
<td>1.1 ± 0.1††</td>
<td></td>
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</tr>
<tr>
<td>FE\textsubscript{Na} (%)</td>
<td>Denervated</td>
<td>2.3 ± 0.2</td>
<td>3.3 ± 0.4</td>
<td>1.1 ± 0.2†</td>
<td>1.6 ± 0.2*</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Innervated</td>
<td>1.2 ± 0.2†</td>
<td>1.5 ± 0.2†</td>
<td>0.4 ± 0.1†</td>
<td>0.8 ± 0.1††</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. cu\textsubscript{UV} and cu\textsubscript{U\textsubscript{Na}\textsubscript{V}} after 40 min of VE in the denervated and innervated kidneys of lean (□) and obese (■) Zucker rats

The cumulative values (in units of µl or µmol·min\(^{-1}\)·g\(-1\) kidney weight) were obtained by summing the absolute values for each collection period from the start to the finish of the VE period, and then subtracting the baseline value. Significance was calculated by adding up the absolute values at each time point from the beginning to the end of VE, and then subtracting the baseline value from this total.

VE did not result in any changes in heart rate or RBF from baseline values in either the lean or the obese animals, while MAP declined gradually after 10 min of VE; the decreases were significant over the last 10 min of the experiment, and were approx. 6% in the lean rats and 8% in the obese rats (both \(P < 0.001\)). Figure 1 presents a comparison of the magnitude of the diuretic and natriuretic responses to VE in the lean and obese Zucker rats, in terms of cumulative \(U_{V}\) (cu\textsubscript{UV}) and cumulative \(U_{Na}\)\(V\) (cu\textsubscript{U\textsubscript{Na}\textsubscript{V}}) over 40 min of VE. The cumulative values were calculated by adding up the absolute values at each time point from the beginning to the end of VE, and then subtracting the baseline value from this total. cu\textsubscript{UV} and cu\textsubscript{U\textsubscript{Na}\textsubscript{V}} after 40 min of VE in the obese rats were significantly lower than those recorded in the lean rats, by 40% and 36% respectively in the denervated kidneys, and by 50% and 46% respectively (all \(P < 0.001\)) in the innervated kidneys. It should be noted that the difference between the basal levels of \(UV\) and \(U_{Na}\)\(V\) in the denervated and innervated kidneys of each group continued throughout the period of VE, with differences in cu\textsubscript{UV} and cu\textsubscript{U\textsubscript{Na}\textsubscript{V}} of 24% and 26% respectively in lean rats, and of 39% and 38% respectively in obese rats (all \(P < 0.001\)).

Protocol 2: effects of L-NAME on renal haemodynamics and excretory functions

L-NAME caused a modest but significant increase in MAP of 4% (\(P < 0.01\)) and 5% (\(P < 0.05\)) in the lean and obese rats respectively (Table 1). This increase was accompanied by a significant fall in heart rate of 6% in the lean and 5% in the obese animals (both \(P < 0.01\)). RBF and GFR in both the denervated and innervated kidneys were unaltered in both groups. In lean animals, there were no significant changes in \(UV\), \(U_{Na}\)\(V\) or FE\textsubscript{Na} in either kidney after L-NAME infusion (Table 1), whereas in the obese rats there were increases of approx. 28% in \(UV\), 20% in \(U_{Na}\)\(V\) and 45% in FE\textsubscript{Na} in the denervated kidneys (all \(P < 0.05\)); the magnitude of the increments was greater still in the innervated kidneys, 66%, 120% and 100%, respectively (all \(P < 0.05\)). The initial values for \(UV\), \(U_{Na}\)\(V\) and FE\textsubscript{Na} in both lean and obese rats were

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Nitric oxide and obesity

Figure 2  cuUV and cuU_{Na}V after 40 min of VE in untreated (■) and L-NAME-treated (■■) lean (A) and obese (B) Zucker rats

Values are given in units of μl or μmol·min⁻¹·g⁻¹ kidney weight. Significance of differences: *P < 0.05, **P < 0.01, ***P < 0.001 compared with untreated rats; †P < 0.001 for denervated compared with the corresponding innervated kidney.

significantly higher for the denervated compared with the corresponding innervated kidney (all P < 0.05), and this difference remained following L-NAME administration.

VE did not significantly alter MAP or RBF in L-NAME-treated lean or obese animals. In lean Zucker rats, MAP in the L-NAME-treated group before VE was significantly higher (P < 0.001) than that in the untreated group, and these differences persisted throughout the remainder of the experiment. In contrast, RBF was not significantly different between the two lean groups either before or during VE. The profiles of MAP and RBF over the course of VE in the obese rats were found to follow the same pattern as in the lean rats. VE in the presence of L-NAME induced a diuresis and natriuresis from both kidneys of lean and obese rats, but this occurred to a greater extent in the innervated kidneys (both P < 0.001, with the result that, by the end of VE, there were no differences in cuUV and cuU_{Na}V between the denervated and innervated kidneys in the L-NAME-treated lean or obese rats (Figure 2).

Basal UV and U_{Na}V were similar in the untreated and L-NAME-treated lean animals before the acute saline challenge. VE induced a significantly (P < 0.05) larger increase in cuUV (by 23%) in the denervated kidneys of L-NAME-treated compared with untreated lean rats, but the increases in cuU_{Na}V did not differ (Figure 2A). However, both cuUV and cuU_{Na}V were augmented in the innervated kidneys of L-NAME-treated compared with untreated lean animals, being elevated by 65% (P < 0.01) and 48% (P < 0.05) respectively. In the obese rats (Figure 2B), L-NAME enhanced cuUV and cuU_{Na}V by 98% and 48% respectively in the denervated kidneys, and by 204% and 136% respectively in the innervated kidneys (all P < 0.001). L-NAME treatment of the obese rats increased the magnitude of the VE-induced diuresis and natriuresis by some 3-fold more than in the lean rats. Most importantly, L-NAME enhanced the renal responses to VE in the obese animals to a level that was not significantly different from the responses obtained in the untreated lean controls.

Protocol 3: characterization of specific NOS isoform

Administration of 7-nitroindazole or aminoguanidine had no effect on MAP, heart rate, RBF or GFR in the obese rats (Table 2). However, in the basal state, UV, U_{Na}V and F_{Na} in the 7-nitroindazole group were significantly (P < 0.05) greater in the denervated kidneys than in the innervated kidneys (Table 2). Moreover, 7-nitroindazole infusion did not alter the elevated excretory rates present in the denervated kidneys. In the aminoguanidine group, renal excretory function was greater in the denervated kidneys (P < 0.01; Table 2), and administration of aminoguanidine significantly increased basal UV, by 53% in the denervated kidneys (P < 0.05) and by 74% in the innervated kidneys (P < 0.01), whereas U_{Na}V increased only on the innervated side. Aminoguanidine infusion enhanced U_{Na}V by 60% (P < 0.05), with a concomitant rise in F_{Na} of 75% (P < 0.01). Although these increments were somewhat larger and occurred mainly in the innervated kidneys, the values of all renal excretory variables after aminoguanidine infusion were still higher (P < 0.01) in the denervated kidneys than in the corresponding innervated kidneys.
There were no significant differences in MAP prior to VE between the obese groups receiving the NOS inhibitors [L-NAME (Table 1), 7-nitroindazole and aminoguanidine (Table 2)], but this was no longer evident once VE was begun. The values and profile of MAP in the aminoguanidine-treated group were similar to those of the L-NAME-treated animals, in which MAP remained relatively constant over the course of VE. However, MAP in the group given 7-nitroindazole began to decline 10 min after starting VE, which was a trend very similar to that noted in untreated obese rats, reaching statistical significance in both groups only in the last 10 min of VE (both \( P < 0.01 \)), at which time it was 7% lower than the pre-VE value. The overall MAP values during the VE period in the 7-nitroindazole-treated and untreated obese groups were significantly lower \( (P < 0.05) \) than those in the L-NAME- and aminoguanidine-treated animals. There were no differences in basal RBF in any of the experimental groups, and this parameter did not change further over the remaining part of the VE experiment.

Comparisons of the cumulative excretory responses to VE between the groups of obese Zucker rats receiving the three NOS inhibitors and the untreated obese rats are illustrated in Figure 3. Acute saline loading raised \( cuU \) and \( cuUnV \) in both kidneys of 7-nitroindazole-treated rats to values that were not significantly different from those in the untreated rats. In the aminoguanidine-treated rats, \( cuU \) and \( cuUnV \) in the denervated kidneys were also comparable with those in the untreated rats, whereas they were both significantly greater in the innervated kidneys \( (P < 0.001 \) for \( cuU \); \( P < 0.01 \) for \( cuUnV \)). However, the increments in these parameters in the aminoguanidine-treated group were still significantly less than those obtained in the L-NAME-treated rats (both \( P < 0.001 \)).

**DISCUSSION**

The present study examined a potential interaction between NO and the renal sympathetic nerves in modulating the ability of the kidney to excrete an acute saline volume load in a rat model of obesity-induced hypertension. The Zucker strain of obese rats was selected because it shares many similarities with obese Zucker rats, cu

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**Table 2** Effects of 7-nitroindazole and aminoguanidine on basal systemic and renal haemodynamics and excretory functions in obese Zucker rats

Values are means ± S.E.M. gkwt, g of kidney weight. Significance of differences: *\( P < 0.05 \), **\( P < 0.01 \) compared with before infusion; †\( P < 0.05 \), ††\( P < 0.01 \) compared with denervated kidney.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Kidney</th>
<th>Before infusion</th>
<th>After infusion</th>
<th>Before infusion</th>
<th>After infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mmHg)</td>
<td>Denervated</td>
<td>115 ± 1</td>
<td>113 ± 1</td>
<td>111 ± 8</td>
<td>116 ± 2</td>
</tr>
<tr>
<td>Heart rate (beats·min⁻¹)</td>
<td>Denervated</td>
<td>371 ± 5</td>
<td>369 ± 7</td>
<td>371 ± 7</td>
<td>369 ± 7</td>
</tr>
<tr>
<td>RBF (ml·min⁻¹·gkwt⁻¹)</td>
<td>Denervated</td>
<td>6.8 ± 0.8</td>
<td>6.6 ± 0.8</td>
<td>6.2 ± 0.5</td>
<td>6.6 ± 0.4</td>
</tr>
<tr>
<td>GFR (ml·min⁻¹·gkwt⁻¹)</td>
<td>Denervated</td>
<td>1.0 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>UV (µl·min⁻¹·gkwt⁻¹)</td>
<td>Denervated</td>
<td>17.1 ± 2.7</td>
<td>18.5 ± 3.6</td>
<td>8.3 ± 1.0</td>
<td>12.7 ± 1.4*</td>
</tr>
<tr>
<td>UnV (µmol·min⁻¹·gkwt⁻¹)</td>
<td>Denervated</td>
<td>2.9 ± 0.3</td>
<td>3.0 ± 0.6</td>
<td>1.3 ± 0.2</td>
<td>1.7 ± 0.3</td>
</tr>
<tr>
<td>FEK (%)</td>
<td>Denervated</td>
<td>1.9 ± 0.3</td>
<td>2.2 ± 0.4</td>
<td>1.1 ± 0.2</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td>Innervated</td>
<td>Denervated</td>
<td>0.7 ± 0.1†</td>
<td>0.7 ± 0.2†</td>
<td>0.5 ± 0.1†</td>
<td>0.8 ± 0.1™†</td>
</tr>
<tr>
<td>Innervated</td>
<td>Innervated</td>
<td>0.5 ± 0.1†</td>
<td>0.6 ± 0.1†</td>
<td>0.4 ± 0.1†</td>
<td>0.7 ± 0.1™†</td>
</tr>
</tbody>
</table>

**Figure 3** cuUV and cuUnV after 40 min of VE in the denervated and innervated kidneys of obese Zucker rats: effects of NOS inhibitors

Open bars, untreated; solid bars, 7-nitroindazole-treated; hatched bars, aminoguanidine-treated; stippled bars, L-NAME-treated. Values are given in units of µl or µmol·min⁻¹·g⁻¹ kidney weight.
humans suffering from hypertension, insulin resistance, hyperinsulinaemia, Type II diabetes mellitus, as well as having a strong genetic component in the transmission of obesity [3]. It was evident from the findings in the present study that the reflex renal responses to VE were blunted in obesity; one component could have been due to a tonic influence of the renal sympathetic control of fluid and sodium excretion, and another to the derangement of endogenous NO production.

The basal values of $UV$ and $U_{Na}V$ for both the innervated and denervated kidneys of obese Zucker rats were found to be lower than those for the corresponding kidneys of the lean rats, despite similar values of RBF and GFR and a higher MAP. This is likely to be due to an increase in renal tubular sodium and water reabsorption, since $FE_{Na}$ was significantly lower in the obese animals. Decreased sodium excretion has been reported previously to occur in association with hypertension in obese humans, fat-fed obese dogs and obese Zucker rats [3,5,6,8,16]. These previous studies also demonstrated that this sodium retention was not mediated by alterations in renal haemodynamics, but appeared to be caused mainly by increased tubular sodium reabsorption, possibly at a site beyond the proximal tubule [5].

There was a greater difference in $U_{Na}V$ between the denervated and innervated kidneys in the obese than in the lean rats, suggesting a greater influence of renal sympathetic nerve control of sodium excretion under resting conditions. Thus the possibility arises that one cause of the augmented sodium reabsorption could have been raised sympathetic activity acting at a tubular level to decrease $UV$ and $U_{Na}V$. This would be consistent with the reports of Kassab et al. [8], who showed that bilateral renal denervation greatly ameliorated sodium retention in fat-fed obese dogs, and of Tuck [15], Hall et al. [16] and Masuo et al. [12] that plasma and urinary catecholamines were increased in obese animal models and obese humans. Further support for this conclusion comes from experiments showing that basal efferent renal sympathetic nerve activity was raised in obese hypertensive fat-fed and Zucker rats [11,13,14].

The depressed sodium and water excretion observed in the obese rats could not have been due solely to the renal sympathetic nerves, since the lowered excretory functions compared with the lean rats were also evident in the denervated kidneys under basal conditions. This impaired renal sodium excretion in obese animals may result from hyperinsulinaemia and insulin resistance. Several studies in humans and experimental animals have shown that insulin, while not affecting RBF or GFR, reduced urinary sodium excretion [37,38]. Moreover, insulin has been reported to stimulate the Na$^+$/P$_i$ symporter and the Na$^+$/H$^+$ antiporter in isolated proximal tubules [37]. Although plasma insulin was not measured in the present study, it is likely that the obese rats had developed insulin resistance and hyper-insulinaemia, as reflected by the observed hyperglycaemia [9].

The small decreases in MAP in both the lean and obese rats during VE might have been due to an interaction between the volume reflex and the arterial baroreflex, as it has been reported that the cardiopulmonary afferent input exerts a tonic inhibitory action on baroreflex sensitivity which is enhanced when the volume reflex is stimulated acutely [39]. The present findings that MAP over the course of the VE was decreased to a similar extent in both obese and lean rats could be taken to indicate that this control mechanism was preserved in the obese animals.

The results of the present study demonstrate that there was a blunted renal excretory response to acute saline VE in the obese Zucker rats compared with the lean rats, in spite of a higher MAP, which strengthens the view that these animals had underlying raised sodium reabsorption. This finding is consistent with other published data showing an attenuated diuretic and natriuretic response to acute oral or intravenous sodium loads in obese rats and dogs [7,9,10]. This deficit in renal sodium handling is also reflected in the observation that an abnormal pressure–natriuresis relationship is present in several models of obese hypertensive animals [3–6,8].

Since obesity is accompanied by overactivity of the sympathetic nervous system, a defect in the neural control of the kidney may have contributed to the blunted diuresis and natriuresis seen in obese animals in the present study, and this was supported by three pieces of data. First, the blunted excretory response in obese rats was more apparent in the innervated than in the denervated kidney; secondly, renal denervation significantly attenuated this blunted response; and thirdly, the obese rats showed a greater disparity between the denervated and innervated kidneys after VE with regard to cu$UV$ and cu$U_{Na}V$. These observations are probably consistent with an inability to suppress renal sympathetic nerve activity during volume loading in this animal model. This was supported by our previous study [14], which used direct recording of the activity of renal nerves to show that the reflex efferent renal sympatho-inhibition to VE was impaired in fat-fed obese rats.

1-NAME infusion raised MAP and decreased heart rate to the same extent in obese and lean rats, whereas basal $UV$, $U_{Na}V$ and $FE_{Na}$ were increased in the obese but not the lean rats, even though RBF and GFR were unchanged. The decreased reabsorption of water and sodium may have been a consequence of inhibiting a direct tubular action of NO, or a suppressive effect of blocking NO on the renal sympathetic nerve control of sodium excretion. The latter possibility was suggested by results showing a greater decrease in excretory function in the innervated kidney. These findings give rise to the notion that NO is involved in the basal control of renal function in obese Zucker rats.
In the lean Zucker rats, L-NAME enhanced the VE-induced diuresis to a greater extent in the innervated kidney than in the denervated kidney. In addition, L-NAME increased the magnitude of the natriuretic response to the saline load in the innervated, but not the denervated, kidney. Furthermore, the differences in UV and $U_{Na}V$ between the denervated and innervated kidneys were not apparent once the volume load was infused. These findings give rise to the view that L-NAME exerts its effect primarily via suppression of the influence of renal sympathetic nerves on renal excretory function. This suggestion is consistent with previous reports showing that NO exerts a renal sympatho-excitatory effect [27–29]. However, the observation that L-NAME promoted a diuresis in the denervated kidney independent of natriuresis implies that NO modulates the renal excretion of water and sodium via separate mechanisms, and that the diuretic response was not the result of inhibition of the renal sympathetic nerve control of fluid excretion. It is possible that this effect could be mediated via an action at the collecting duct, or by alterations in the secretion and/or action of antidiuretic hormone.

In the obese rats, VE in the presence of L-NAME resulted in a marked increase in diuresis and natriuresis compared with the response in the untreated rats. This enhanced diuretic and natriuretic effect following L-NAME was observed in both the denervated and innervated kidneys, but to a greater degree in the innervated kidney, compatible with greater sympatho-inhibition during L-NAME infusion. Supporting evidence for this view came from the observation that there were no differences between the denervated and innervated kidneys with respect to $cuUV$ and $cuU_{Na}V$ at the end of VE. Although MAP was higher in the L-NAME group than in the untreated group throughout the VE period, it was only slightly raised, and is unlikely to have accounted for the markedly enhanced renal excretory response. In addition, there was no difference in RBF between the L-NAME-treated and untreated groups at any point during VE, and thus it is unlikely that these effects of L-NAME were mediated by changes in renal haemodynamics. Taken together, it is reasonable to suggest that the enhanced diuretic and natriuretic responses in the presence of L-NAME may be mediated by inhibition of a direct tubular action of NO and its interaction with the renal sympathetic nerves.

There were no measurable effects on basal systemic and renal haemodynamics or renal excretory function in obese rats given 7-nitroindazole. These observations suggest that 7-nitroindazole most probably has negligible effects on eNOS, and give rise to the possibility that NO originating from nNOS may not play a role in controlling systemic/renal haemodynamics or renal excretory function in the resting state. Likewise, because MAP, RBF and GFR were relatively stable during aminoguanidine infusion, it is unlikely that this inhibitor was affecting eNOS. However, aminoguanidine increased $cuUV$ in both kidneys, whereas it raised $cuU_{Na}V$ only in the innervated kidney. Again, these observations could be interpreted as supporting the view of independent regulation of water and sodium by NO, by direct tubular action on the one hand and via a sympatho-excitatory effect on the other. Moreover, these data lend credence to the possibility that NO generated by iNOS may be involved to some extent in the basal control of renal excretory function in the obese animals.

The magnitude of the diuretic and natriuretic responses to VE in the 7-nitroindazole-treated obese rats was comparable with that in the untreated rats, but was smaller than those in the L-NAME group in both the denervated and innervated kidneys. This was unlikely to have been due to the lower MAP in the 7-nitroindazole group, since RBF was similar to that in the L-NAME group; this was also the case in the aminoguanidine group, which has similar systemic and renal haemodynamics. These findings indicate that prevention of the generation of NO by nNOS is unlikely to have been responsible for the enhanced renal responses to VE in the presence of L-NAME. In the case of aminoguanidine, although the magnitude of the VE-induced diuresis and natriuresis was lower than in the L-NAME-treated group, that in the innervated kidney was greater than in the untreated rats. This suggests that part of the enhanced renal response to VE in the innervated kidney of the L-NAME-treated rats was mediated by the inhibition of NO originating from iNOS, and was renal-nerve-dependent.

In conclusion, the results of the present study suggest that endogenous NO production is increased in obesity, and might contribute to the depressed ability to excrete a saline load. The NO responsible for this abnormality appears to be generated by the eNOS and iNOS isoforms of the enzyme. The effect of NO produced by iNOS seems to be determined by the background level of renal nerve activity, whereas the eNOS-generated NO appears to exert its effect independently of the renal innervation status. Finally, blocking NO synthesis by L-NAME corrected the blunted volume reflex in obese Zucker rats.

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