Renal contribution to thermolability in rats: role of renal nerves

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Abstract

Background. Body temperature is closely regulated via the integration of a number of mechanisms, the study of which has been greatly assisted by the exploitation of comparative physiology. Previous studies have demonstrated that chronic renal failure patients have significantly lower body temperatures than healthy subjects when artifacts from circadian changes were taken into consideration. We hypothesize that the blunting of renal sensory neurons after kidney partial ablation may contribute to the lack of suppression of sympathetic efferent outflow towards BAT, modifying the glucose metabolism signaling pathway, UCP1 expression and liver mitochondrial respiratory chain activity.

Methods. To evaluate the influence of renal mass reduction, renal denervation and chronic deafferentation by capsaicin on thermoregulation, glucose metabolism, UCP1 expression and liver mitochondrial respiration, was used respectively, the blocking of heat dissipation by thermoneutral body water immersion, the oxygen consumption by Clark-type electrode, and western blot method.

Results. The study confirmed that, following 5/6 nephrectomy, the basal core temperature of rats was significantly lower than that of control animals when maintained in a thermoneutral body water immersion recipient, as compared to controls. Additionally, we demonstrated that exposure of bilateral renal denervated or of renal chronic capsaicin-treated rats to a similar experimental protocol results in a fast and high rise in rectal temperature response, and this is associated with a significant increase in the basal serine phosphorylation and protein levels of Akt and protein levels of UCP1. This was observed despite unchanged liver mitochondrial respiratory control and ADP/O ratios in 5/6Nx, as well as DNx, when compared to control mitochondria.

Conclusions. Speculatively, it may be suggested that one of the renal sensory nerve signal defects associated with decreased kidney energy generation, induced by kidney ablation, may result in an inability to control the body temperature.

Keywords: kidney; partial nephrectomy; temperature regulation; UCP

Introduction

The body constantly produces heat (energy) and exchanges it with the environment. Body temperature is kept constant if energy gain equals energy loss [1,2]. If energy gain does not equal energy loss, the extra heat is ‘stored’ or lost from the body. Aschoff (reviewed in [3]) was the first to suggest that one should differentiate between body core temperature, which is maintained around 37°C, and body shell temperature, which depends largely on the environmental temperature [3]. In order to regulate body core and shell temperature, the mammal brain coordinates a range of behavioural and autonomic control mechanisms [4,5].

Mammals are homeothermic organisms and body temperature regulation is tight as this is essential for cell functioning. Body temperature is closely regulated via the integration of a number of mechanisms, the study of which has been greatly assisted by the exploitation of comparative physiology [4,5]. A previous study [6] demonstrated that chronic renal failure (CRF) patients, in the predialysis stage, have significantly lower body temperatures than healthy subjects when artifacts from circadian changes were taken into consideration. Other authors confirmed that the kidneys play a role in thermogenesis and that nephrectomized rabbits have greater thermolability, lower core temperature and lower tolerance to heat than control animals [7,8]. Recently, subtotal renal mass ablation was found to induce sympathetic nervous system modulation of ventromedial hypothalamic (VMH) nuclei and brown adipose tissue (BAT), which are involved in thermogenesis [9,10].

Thermogenesis is constituted by facultative (FT) and obligatory (metabolic) thermogenesis (OT) [10]. The principal source of heat generation for FT in rodents occurs due to activation of BAT; increasing the activities of uncoupling proteins (UCP) [10–12]. Recently, studies have revealed that mitochondrial UCP play an important role as mediators of non-shivering thermogenesis, and linkage was detected between FT, ucp1 and insulin receptor substrate (IRS1/2) activation after cold stimulation [13]. Following
tyrosine phosphorylation, the IRSs act as docking proteins for several Src homology 2 domain-containing proteins, including phosphatidylinositol 3-kinase (PI 3-kinase), Grb2, SHP2, Nck and Fyn. Downstream to PI 3-kinase, there is activation of a serine/threonine kinase, Akt, which is directly involved in the cell glucose transport-signalling pathway. On the other hand, downstream to Grb2, activation of the mitogen-activated protein kinase (MAPK-ERK) occurs; this kinase is important in the regulation of gene expression and cell growth [14–18].

The OT is mostly associated with the maintenance of the osmotic gradient between the matrix and the internal membrane of the mitochondria, which is formed by the respiratory chain, oxidative phosphorylation and the rate of ATP hydrolysis of the phosphorylated kidney enzymes [11–13,19–22]. In the kidneys, a highly aerobic and heat-generating tissue [contributes to >10% of total body energy (heat) generation], >60% of cellular oxygen consumption may be directed towards supporting the ATP demands of the ATPase-mediated ion transport. The remaining mitochondrial respiratory capacity utilized under conditions of spontaneous respiration is presumably divided between ATP production, as well as non-phosphorylating respiration cellular processes [23]. Our laboratory [24,25] has previously shown that renal sensory neurons are elicited by mechano- and chemoreceptor stimuli. Since the entire physiological significance of renal sensory receptors remains incompletely known, we hypothesized that renal afferent neurons towards the central nervous system (CNS) may neuromodulate many other responses to different stimuli, such as body core temperature changes.

Taking these studies together, we hypothesize that the blunting of renal sensory activity after kidney partial ablation may contribute to the lack of suppression of sympathetic efferent outflow towards BAT, modifying the glucose metabolism signalling pathway, UCP 1 expression and liver mitochondrial respiratory chain activity. To test this hypothesis, the present study evaluated the influence of renal mass reduction and renal denervation and renal chronic deafferentation by capsaicin treatment on thermoregulation, using the blocking of heat dissipation by thermoneutral body water immersion in anaesthetized rats randomly assigned to one of the following groups: (1) sham-operated (Sham, n = 5), (2) bilateral renal-denervated (DNx, n = 5), (3) capsaicin-treated (CPS, n = 5), (4) 5/6 nephrectomized rats (5/6 Nx, n = 5) and (4) control, non-operated animals (Co, n = 5).

Material and methods

Experimental design

The experiments were conducted on age-matched, male offspring of sibling-mated Sprague Dawley rats (200–250 g) allowed free access to water and normal rat chow. The general guidelines established by the Brazilian College of Animal Experimentation (COBEA) were followed throughout the investigation. Our local colonies were originated from the breeding stock supplied by the CEMIB/Unicamp, Campinas, SP, Brazil. Immediately after weaning, at 3 weeks of age, animals were maintained under controlled temperature (25°C) and lighting conditions (0700 h–1900 h), with free access to tap water and standard rodent laboratory chow (Nuvital, Curitiba, PR, Brazil) Na⁺ content: 135 ± 3 µEq/g; K⁺ content: 293 ± 5 µEq/g) and studied from 8 to 10 weeks of age.

Surgical procedures

For the renal bilaterally denervated group (DNx, n = 5), surgery was performed prior to the start of the entire 10-week metabolic studies. Briefly, the animals were anaesthetized with an intraperitoneal (i.p.) injection of sodium pentobarbital (50 mg/kg body weight), and after loss of corneal and pedal reflexes, the animals were anaesthetized and both kidneys were exposed through dorsal abdominal incisions and surgically denervated with the aid of a stereomicroscope. Denervation was accomplished by cutting all visible nerves along the renal artery and by stripping the connective tissue passing next to and along the course of the renal artery and vein. Immediately thereafter, the renal vessels were surrounded with cotton swabs previously soaked in 10% (v/v) phenol diluted in absolute ethanol [26,27]. Observations were made of rats individually housed in metabolic cages located in a room with controlled temperature, a 12-h photoperiod and humidity. The rats in each group were fed a uniform amount of pel- let chow on a given day. The animals had free access to tap water throughout the 10-week observation period. For capsaicin treatment (chronic capsaicin deafferentation), rats were treated with subcutaneous injections of capsaicin solution (12.5 mg/ml dissolved in vehicle). The total dose of capsaicin (125 mg/kg) was divided into three injections (25 mg/kg in the morning and 50 mg/kg in the afternoon on the first day and 50 mg/kg on the second day). The control group received vehicle treatment (10% tween 80, 10% ethanol and 80% saline) under identical conditions of administration. Experiments were performed 10 days later in rats that showed the disappearance of the corneal chomosensory reflex (eye wiping for 1–3 min in response to a drop of 0.1% ammonium hydroxide instilled in one eye) 24 h before the study [28].

The renal mass reduction group (5/6 Nx) is a widely used model of chronic renal insufficiency that is associated with a progressive glomerular sclerosis and gradually declining glomerular filtration rate [29]. Briefly, after anaesthetizing with sodium pentobarbital i.p., incisions were made in the right and left flanks. The right kidney was removed, preserving the adrenal gland. Both poles from the left kidney were carefully excised. The rats were used for experimentation 1 week after surgery.

For the passive body heating group, achieved by blocking heat dissipation during thermoneutral body water immersion, a previously described model of passive heating [3] was used. Briefly, after immersing the whole body (except head) in warm water, the temperature of which was similar to that of the rat rectal temperature, the heat dissipation was blocked by thermal isolation. Immediately, the rectal temperature was continuously recorded in sodium pentobarbital anaesthetized rats every minute for 30 min using a thermal probe (Harvard, Boston, USA) maintained 5 cm in the rectum with a sensitivity graded in hundredths (±0.01°C) of Celsius degrees (IOPE, São Paulo, Brazil). The thermal
curves were performed 7 days after the surgical procedure for chronic kidney injuries, kidney denervation procedures (5/6 Nx and DNx) or 10 days after CPS treatment, and the groups were submitted to the body thermoneutral water immersion protocol using a water bath (Bioetica, São Paulo, Brazil) and compared with the Sham group. The data from all groups were plotted to compare the following parameters: initial and final temperature, and slope and area under curve (AUC). To characterize whether the specific sympathetic β-adrenoreceptor is involved in energy generation and in the body temperature control of 5/6 Nx and DNx models, the animals were previously treated i.p. with either saline (NaCl 0.15 M) or the specific β-adrenoreceptor blocker, 20 mg/kg b.w. Propranolol (Sigma Chem. Co., St Louis, MO, USA).

Liver mitochondria isolation

Forty-eight hours after 0.25 M NH₄Cl feeding, mitochondria were isolated from the livers of adult Wistar rats by conventional differential centrifugation. Briefly, rat livers were rapidly removed, finely minced and homogenized in an ice-cold buffer containing 250 mM sucrose, 0.5 mM EGTA and 10 mM HEPES buffer (pH 7.2). The mitochondrial suspension was then centrifuged at 500 g for 7 min, the resulting supernatant was centrifuged at 7800 g for 10 min and the pellet was resuspended in the EGTA-free buffer at 6000 g for 10 min. Mitochondrial protein concentration was determined by the Biuret method, modified by the addition of cholate. The experiments were performed at 28 °C in a standard medium containing 125 mM sucrose, 65 mM KCl, 1 mM MgCl₂, 2 mM Pi, 10 mM HEPES and 5 mM succinate (pH 7.2) and 17 μM Ca²⁺, as determined by atomic absorption. Oxygen consumption experiments were performed in this same standard medium in the presence of 0.5 mM EGTA (see reference [30] for details).

Measurements of mitochondrial respiration

Oxygen consumption was measured in a 1.3 ml thermostated water-jacketed vessel equipped with a magnetic stirrer, using a Clark-type electrode (Yellow Spring Instruments Company) connected to a recorder. Rat liver mitochondria (RKM, 0.5 mg/ml) were added to the standard reaction medium at 28 °C. Respiration rates are given in nmol O₂ min⁻¹ mg protein⁻¹. Phosphorylating (state III) respiration was initiated by the addition of 200 nmol ADP/mg protein. Phosphorylation efficiency (ADP/O ratio) was calculated from the amount of ADP added and the total amount of oxygen consumed during state III. To study the effect of metabolic acidosis on UCP activity, the mitochondria respiration was tested in the presence of CAT (carboxiatriltoside), GDP (guanosine di-phosphate) and BSA (bovine serum albumin) (see reference [30] for details).

Western blot

Seven days after the surgical kidney procedures, animals of all groups were sacrificed by cervical dislocation. The rats were anaesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight), and used as soon as anaesthesia was assured by the loss of pedal and corneal reflexes. The BAT was excised and quickly removed by cervical incisions, minced coarsely and homogenized immediately in a solubilization buffer containing 100 mM Tris (pH 7.6), 1% Triton X-100, 150 mM NaCl, 0.1 mg aprotinin, 35 mg PMSF/ml, 10 mM Na₃VO₄, 100 mM NaF, 10 mM Na₃P₂O₇ and 4 mM EDTA, using a Polytron PTA 20S generator operated at a maximum speed for 30 s and clarified by centrifugation. Invariably, the tissue samples were pooled and 200 μg protein were used as whole tissue extract for PI3k, Akt and Erk analysis or equal amounts of protein for immunoprecipitation, followed by western blot for IRS-1 with the indicated antibodies and [¹²⁵I] Protein A. Quantitative analyses of the blots were performed using Scion Image software. [¹²⁵I] Protein A, bound to the anti-phosphotyrosine and antipeptide antibodies, was detected by autoradiography using preflashed Kodak XAR film (Eastman Kodak Co., Rochester, NY, USA) with Cronex Lightning plus intensifying screens at −80°C for 12–48 h. The antibodies against IRS-1 (sc-559), Erk (sc-93), p-Erk (sc-7383), Akt1 (sc-1618) and anti-phosphotyrosine (sc-508), PI3k and UCPI were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA) [31,32].

Catecholamine assay

Plasma catecholamines were extracted from the medium using Al₂O₃ (alumina) and HBA (dihydroxybenzylamine) as internal standard, and quantified by ion-pair reverse phase chromatography coupled with electrochemical detection (0.5 V) as described by Di Marco et al. [33]. Kidney norepinephrine contents were assessed by the Anton and Sayre method [34].

Statistical analysis

For comparison, the results from at least three independent experiments were performed in triplicate using mitochondria isolated from that animal. Protein phosphorylation values from control and experimental groups were calculated and expressed as Δ%. All data are reported as means ± SD and analysed using appropriate ANOVA or Mann–Whitney tests. Post hoc comparisons between selected means were performed with Bonferroni’s contrast test when initial ANOVA indicated statistical differences between experimental groups. Comparisons involving only two means within or between groups were made using a Student’s test. A P value < 0.05 was considered to indicate significance.

Results

Figure 1A and B and Table 1 show the effects of thermoneutral body water immersion on body core temperature values in control, denervated and partial nephrectomized rats, expressed as means ± SEM.

Temperature measurements

At the onset of registering the temperature in the laboratory environment temperature (25°C), CPS, DNx and Sham rats...
had similar temperatures (37.05 ± 0.11°C, 37.81 ± 0.26°C and 36.92 ± 0.16°C, respectively) (Table 1). However, the basal core temperature of 5/6 Nx rats (35.10 ± 0.30°C) was significantly lower than those of the CPS, DNx and Sham groups (P ≤ 0.05). Exposure of animals to passive body heating by blocking heat dissipation in thermoneutral water immersion led to a significant and rapid increase in the average core temperature change in DNx and CPS rats, reaching higher final temperature values compared to Sham and 5/6 Nx groups (Figure 1A, C and Table 1). Despite the lower core temperature in 5/6 Nx after 30 min, the thermal gain in these animals was similar to that observed in Sham animals (Figure 1B, Table 1). Figure 1B depicts the increased calculated area under the temperature curve in 5/6 Nx rats, compared with other groups, suggesting a capacity of these animals to maintain caloric generation and body thermal homeostasis. None of the temperature parameters were different between control (non-operated rats) and Sham groups; however, pre-treatment with Propranolol (Prop), a specific β-adrenoreceptor blocker, abolished the decreased basal core temperature observed in 5/6 Nx rats and the sudden increase in the average core temperature registered in DNx rats, when compared to untreated groups after 30 min passive body heating (Co+Prop: 38.64 ± 0.47°C, n = 6; Sham+Prop: 39.43 ± 0.62°C n = 7; 5/6 Nx+Prop: 38.53 ± 0.33°C, n = 6; DNx+Prop: 38.34 ± 0.37°C, n = 7; P ≤ 0.02), as shown in Table 1.

Measurements of mitochondrial respiration

Figure 2 shows succinate-supported respiration and the efficiency of oxidative phosphorylation in liver mitochondria isolated from control, denervated and 5/6 Nx-treated rats. No difference was observed in the resting respiration (state IV) of liver mitochondria isolated from the controls, compared to DNx and 5/6 Nx rats (Figure 2). Accordingly, respiratory control values were similar in DNx (RC = 2.6), 5/6 Nx (RC = 2.6) mitochondria and in control mitochondria (RC = 3.2). In addition, no differences could be detected in respiratory control and the ADP/O ratio in liver mitochondria from the different groups (Figure 2). The ADP/O ratios were similar in all groups, indicating that the respiratory chain activity was not affected by the different treatments.

**Table 1.** Results of thermal curves performed 7 days after the surgical procedure for chronic kidney injuries or kidney denervation procedures (5/6 Nx and DNx) and 10 days after capsaicin treatment compared with the Sham group. The data of all groups were plotted to compare the initial and final temperatures and area under curve (AUC). To characterize whether the specific sympathetic β-adrenoreceptor is involved in the body temperature control of 5/6 Nx and DNx models, the animals were previously treated with 20 mg/kg propranolol.

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>5/6 Nx</th>
<th>DNx</th>
<th>CPS</th>
<th>5/6 Nx+Prop</th>
<th>DNx+Prop</th>
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<tr>
<td>Initial T (°C)</td>
<td>36.9 ± 0.16</td>
<td>35.1 ± 0.30&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td>37.1 ± 0.26</td>
<td>37.6 ± 0.59</td>
<td>36.14 ± 0.73</td>
<td>36.64 ± 0.36&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Final T (°C)</td>
<td>39.1 ± 0.22</td>
<td>38.7 ± 0.56&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.4 ± 1.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.7 ± 0.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.53 ± 0.33</td>
<td>38.34 ± 0.37</td>
</tr>
<tr>
<td>AUC</td>
<td>41.0 ± 3.06</td>
<td>63.9 ± 13.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45.2 ± 4.62</td>
<td>55.0 ± 6.61</td>
<td>45.22 ± 4.62</td>
<td>57.05 ± 17.82</td>
</tr>
</tbody>
</table>

AUC: area under curve.
The data are reported as the means ± SD.
<sup>a</sup>versus Sham and <sup>b</sup>versus DNx to P < 0.05 (ANOVA).
mitochondria isolated from controls and experimental groups (Figure 2).

**Effect of partial nephrectomy and renal denervation on tyrosine phosphorylation of IRS-1 and MAP kinase (Erk-2) and serine phosphorylation of PI 3-kinase and Akt in the BAT**

BAT from control, 5/6 Nx or DNx-treated rats were submitted to immunoprecipitation with the α-IRS-1 antibody and then blotted with the anti-phosphotyrosine antibody (Figure 4). The BAT basal levels of IRS-1 tyrosine phosphorylation were significantly higher in partially nephrectomized rats (120.6 ± 26.09%) compared to all the other groups (Co: 100%; Sham: 62.91 ± 2.39% and DNx: 97.73 ± 5.75%, with P ≤ 0.001). The protein expression of IRS-1 in the BAT of 5/6 Nx was quantitated by immunoprecipitation and immunoblotting with α-IRS-1 antibodies. Protein levels of IRS-1 were also enhanced compared to controls and the renal denervated groups (Figure 4). The basal levels of serine phosphorylation and protein levels of PI 3-kinase and MAP kinase (Erk-2) in the BAT were not significantly different between groups (data not shown). As shown in Figure 3, the basal serine phosphorylations of Akt in 5/6 Nx and DNx rats were equally higher in both groups (5/6 Nx: 116.2 ± 3.04%; DNx: 116.2 ± 2.47%, P = 0.07), compared to control rats (Co: 100% and Sham: 101.5 ± 4.95%), with P ≤ 0.05. The BAT protein levels of Akt were also significantly different between 5/6 Nx and DNx groups, compared to controls (Figure 3). In order to ascertain a possible involvement of uncoupling proteins (UCP 1) in the results obtained, western blots for UCP 1 were performed in BAT isolated from all groups. Figure 5 shows that the basal protein levels of UCP 1 in the BAT were significantly higher in the 5/6 Nx and DNx groups, compared to Co and Sham animals (P ≤ 0.01).

**Catecholamine quantification**

The present study shows a significant change in the norepinephrine (NE), epinephrine (EPI) and dopamine (DA) plasma levels in the 5/6 Nx and renal NE levels in the DNx kidney tissue when compared to Sham rats. As shown in Table 2, the plasma levels of NE (P < 0.001), EPI and DA (P < 0.05) levels were higher in the 5/6 Nx than in the Sham rats. The effective renal denervation was confirmed by decreased renal NE concentration in DNx when compared to non-denervated kidneys, (P < 0.001).

**Discussion**

This study was designed to evaluate the effect of renal mass reduction, renal chronic deafferentation and renal bilateral denervation on thermolability, the BAT cell glucose transport signalling pathway, UCP 1 expression, plasma...
and colleagues, however, reported that the kidney increases $O_2$, while it reduces the glomerular filtration rate associated with decreases in $T_{Na}$, with, consequently, a sharp reduction in $T_{Na}:Q_2$ [40]. Thus, enhanced mitochondrial $O_2$ usage is associated with an increase in the rate of ATP hydrolysis, which in turn is accompanied by an increased sodium renal tubule efflux [21]. Therefore, in the kidneys, a highly aerobic and heat-generating tissue, the elevated (>60%) cellular oxygen consumption may be directed towards supporting the ATP demands of the ATPase-mediated ion transport tubule; these data may suggest that the organ may contribute to >10% of the whole body energy (core temperature) generation. Thus, the decrease in body temperature observed in the present study may suggest that partial renal ablation, such as that observed in CRF patients, associated with increased thermolability, with greater susceptibility to heat-related disorders, at least in part, could be caused by decreased tubule cell oxygen consumption as a consequence of the fall in the rate of ATP hydrolysis. Surprisingly, in the present study, the partial renal ablation followed by warm water immersion did not affect the body’s capacity for heat generation and, consequently, enhanced the core temperature.

The precise mechanism and influence of the kidneys on body thermogenesis is an unexplored field of research. However, results of the present investigation, showing a rapid and higher rise in rectal temperature response in bilateral denervated and capsaicin-treated rats, suggest the involvement of renal nerve activity in the thermoregulatory set point. We and others [24,25] have previously shown that renal sensory neurons are elicited by mechano- and chemoreceptor stimuli. Since the physiological significance of renal sensory receptors remains incompletely understood, we hypothesized that renal afferent neurons towards the CNS may neuromodulate many other responses to different stimuli, including the body core temperature control.

Body core thermosensors are concentrated in the hypothalamus, but afferent thermal sensor receptors are also located at other core sites, including the midbrain, medulla, spinal cord, cortex and deep abdominal structures, including the kidneys [41,42]. These temperature receptors transmit their information through afferent nerves to the brainstem, especially to the pre-optic/anterior hypothalamus [41,42]. Neurons of the pre-optic/anterior hypothalamus have a key function in coordinating many effector mechanisms by different connections. The present report shows higher plasma catecholamine levels in 5/6 $N_x$ (Table 2), supporting findings of previous studies demonstrating that, in CRF models, there is a reduced autoinhibition of norepinephrine (NE), release limited to specific brain regions such as the anterior hypothalamus, associated with the increased NE turnover rate [43] and excessive NE secretion from posterior hypothalamus [43–45], causing a hyperactive state in the peripheral sympathetic nervous system [44–46], including the kidneys. The central neural signals for thermal modulation, produced by the midbrain, are transmitted to BAT through the inferior olive and intermediolateral cell column, by effector sympathetic neural pathways. In support of this evidence, the results of the present study showed the sudden increase in the average core final temperature in $D_Nx$ rats previously treated with $\beta$-adrenoreceptor blocker and

<table>
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<th>Groups</th>
<th>catecholamine</th>
<th>Norepinephrine</th>
<th>Epinephrine</th>
<th>Dopamine</th>
</tr>
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<tr>
<td>Sham (plasma, 5/6 $N_x$)</td>
<td>113 ± 13.0</td>
<td>111.3 ± 24.85</td>
<td>123.0 ± 16.37</td>
<td></td>
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<tr>
<td>Sham (kidney, $\mu$g/g of tissue)</td>
<td>210.6 ± 41.2</td>
<td>–</td>
<td>–</td>
<td></td>
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<tr>
<td>$D_Nx$ (kidney, $\mu$g/g of tissue)</td>
<td>84.3 ± 28.7a</td>
<td>–</td>
<td>–</td>
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</table>

The data are reported as the means ± SD of $n = 5$ for each group.

aVersus Sham to $P \leq 0.001$ (Mann–Whitney test).
bVersus Sham to $P \leq 0.05$.

catecholamine levels and liver mitochondrial respiratory chain activity in rats, using passive body heating by the thermoneutral water immersion method in anaesthetized rats. In the current study, we confirmed that, following 5/6 nephrectomy, the basal core temperature of rats was significantly lower than that of control animals, and this is associated with a significant increase in the basal serine phosphorylation and protein levels of Akt and protein levels of UCP 1. This was observed despite unchanged liver mitochondrial respiratory control and ADP/O ratios in 5/6 $N_x$, as well as $D_Nx$, when compared to control mitochondria. However, the thermal gain in these animals, when maintained in a thermoneutral body water immersion recipient, was similar to that observed in Sham animals (Figure 1B, Table 1). Additionally, we demonstrated that exposure of bilateral renal denervated or of renal chronic capsaicin-treated rats to a similar experimental protocol results in a fast and high rise in rectal temperature response.

The pathophysiological factors behind this increased thermolability are unknown. Authors have reported the presence of factors in urine that affect body temperature [35]. Knochel and Seldin [36] hypothesized that cyanate might be the toxin responsible for causing the decrease in body temperature associated with CRF models. Other authors, however, have shown that the plasma concentration of cyanate found during uraemia is not high enough to affect the body temperature of rabbits [7,37]. It may be speculated that the substance retained by kidney dysfunction may interfere in hypothalamic areas involved in temperature modulation; however, Silverblatt et al. [38] and Kluger et al. [35] have shown that anephric rabbits respond to pyrogen administration by increasing the body temperature. These data suggest that the central nervous areas, involved in the development of fever, are not impaired during CRF.

On the other hand, the OT is mostly associated with the maintenance of osmotic gradients between the matrix and the internal membrane of the mitochondria, which is formed by respiratory chain, oxidative phosphorylation and the rate of ATP hydrolysis of the phosphorylated kidney enzymes [11–13,19–22]. Since renal oxygen usage ($Q_2$) normally increases linearly with tubule sodium reabsorption ($T_{Na}$) above a basal level [39], the slope of this line ($T_{Na}:Q_2$) defines the efficiency with which the kidney uses $O_2$ for chemical work, above a basal level. Laycock
in chronically capsaicin-treated rats when compared to untreated groups after 30 min of passive body heating. Taking these studies together, we hypothesize that the decreased renal sensory neuron activity after partial kidney ablation may contribute to the lack of suppression of sympathetic efferent outflow towards BAT, modifying glucose metabolism signalling pathways (protein expression and phosphorylation of Akt) and enhancing the UCP1 expression, without any change in mitochondrial respiratory chain activity, compared to control animals. Thus, we may postulate that attenuated afferent renal tone, induced by partial nephrectomy, could promote BAT sympathetic nerve hyperactivity, eliciting an increase in cell glucose influx; furthermore, mitochondrial UCP activity may play a compensatory role as a mediator of non-shivering thermogenesis. However, the significantly lower basal core temperature of 5/6 Nx rats than those of the CPS, DNx and Sham groups and the faster increase in the average core temperature in DNx and CPS rats compared to the Sham and 5/6 Nx groups exposure to passive body heating could not be explained by attenuated afferent renal nerve tone only. This phenomenon must be associated at least in part, with increased thermobility and/or decreased kidney energy (heat) generation in partial nephrectomized rats caused by decreased tubule cell oxygen consumption and a fall in the rate of ATP hydrolysis.

Perspectives

Although the precise mechanism responsible for the thermobility response, observed in partially nephrectomized rats, is still unclear, the remarkable findings of the present investigation lead us to hypothesize that renal neural pathways may play an instrumental role in body thermal homeostasis, resulting in a significant and compensatory sympathetic nervous system overexcitability in BAT, despite decreased kidney energy (heat) generation in partial nephrectomized rats. Speculatively, it may be suggested that one of the renal sensory nerve signal defects, induced by partial nephrectomy, may result in an inability to control the body temperature.

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Conflict of interest statement. None declared.

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