Centrally Administered Resistin Enhances Sympathetic Nerve Activity to the Hindlimb but Attenuates the Activity to Brown Adipose Tissue

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Resistin, an adipokine, is believed to act in the brain to influence energy homeostasis. Plasma resistin levels are elevated in obesity and are associated with metabolic and cardiovascular disease. Increased muscle sympathetic nerve activity (SNA) is a characteristic of obesity, a risk factor for diabetes and cardiovascular disease. We hypothesized that resistin affects SNA, which contributes to metabolic and cardiovascular dysfunction. Here we investigated the effects of centrally administered resistin on SNA to muscle (lumbar) and brown adipose tissue (BAT), outputs that influence cardiovascular and energy homeostasis. Overnight-fasted rats were anesthetized, and resistin (7 μg) was administered into the lateral cerebral ventricle (intracerebroventricular). The lumbar sympathetic nerve trunk or sympathetic nerves supplying BAT were dissected free, and nerve activity was recorded. Arterial blood pressure, heart rate, body core temperature, and BAT temperature were also recorded. Responses to resistin or vehicle were monitored for 4 h after intracerebroventricular administration. Acutely administered resistin increased lumbar SNA but decreased BAT SNA. Mean arterial pressure and heart rate, however, were not significantly affected by resistin. BAT temperature was significantly reduced by resistin, and there was a concomitant fall in body temperature. The findings indicate that resistin has differential effects on SNA to tissues involved in metabolic and cardiovascular regulation. The decreased BAT SNA and the increased lumbar SNA elicited by resistin suggest that it may contribute to the increased muscle SNA and reduced energy expenditure observed in obesity and diabetes. (Endocrinology 152: 2626–2633, 2011)

Resistin is a newly discovered polypeptide, originally identified in adipose tissue (1) but now known to be expressed in smaller amounts in a variety of tissues including the hypothalamus (2, 3). It belongs to a family of cysteine-rich proteins capable of inducing insulin resistance. The plasma levels of resistin are reported to be increased with obesity and type 2 diabetes (1, 4–9), and together with its influence on insulin sensitivity, it has been suggested that resistin may be a potential link between type 2 diabetes and obesity (1). Resistin may also have a role in the regulation of metabolism by decreasing food intake, and its expression is influenced by dietary intake. Plasma levels of resistin are reduced by fasting but are increased with feeding (1). These dietary-induced changes in plasma resistin levels are mediated by glucose and insulin (4).

It is believed that resistin can act within the central nervous system, and it has been detected in cerebrospinal fluid of humans (10). Injections into the cerebral ventricles of rodents decreases food intake and alters the expression of neurotransmitters such as neuropeptide Y and agouti-related peptide, which are decreased, and cocaine- and amphetamine-regulated transcript and proopiomelanocortin, which are increased, in the hypothalamus (11, 12). The findings suggest this brain region may be an important site of action of resistin (13).

Abbreviations: BAT, Brown adipose tissue; icv, intracerebroventricular; SNA, sympathetic nerve activity.
Resistin is associated with cardiovascular disease, including heart failure and hypertension (4–9, 14–17). A characteristic of both heart failure and hypertension is an elevation of sympathetic nerve activity (SNA). SNA to the skeletal muscle vasculature is also elevated in obese individuals and is correlated to abdominal visceral adiposity (18). This may contribute to the cardiovascular complications observed in obesity, which is a recognized risk factor for cardiovascular disease, hypertension, and type 2 diabetes.

Obesity is a complex condition in which there may be metabolic dysfunction and an imbalance between energy intake and expenditure. Brown adipose tissue (BAT) is important in thermogenesis and energy expenditure, and these can be dramatically influenced by alterations in SNA to BAT. Resistin has important effects on energy metabolism (19) but its effects on SNA to BAT are not known.

Because the effects of resistin on SNA have not been investigated to date, the primary aims of the present study were to determine the acute effects of resistin on SNA to the skeletal muscle vasculature (lumbar SNA) and to BAT (BAT SNA), end organs that are important in cardiovascular and energy homeostasis. Blood pressure and heart rate as well as body core temperature and BAT temperature were also monitored. We also investigated the distribution of the protein Fos, a marker of increased neuronal activity, in the hypothalamus to gain an insight into the potential central sites of action of resistin.

Materials and Methods

Animals

All experimental protocols were performed in accordance with the Prevention of Cruelty to Animals Act 1986 (Australia). These protocols conform to the Guiding Principles for Research Involving Animals and Human Beings (2) and the guidelines set out by the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, 2007 (National Health and Medical Research Council of Australia) and were approved by the Royal Melbourne Institute of Technology University Animal Ethics Committee. Male Sprague Dawley rats were obtained from Monash Animal Services. Rats were housed at 23 C with a 12-h light, 12-h dark cycle and allowed free access to standard rat chow and water.

Procedures

General

Rats were fasted overnight before the experiment, because resistin reportedly activates more central neurons in the hypothalamus than in nonfasted rats, suggesting its actions may be more pronounced in fasted rats (20). On the day of the experiment, anesthesia was induced using isoflurane gas (2.5–3%) in O2, and then a catheter was inserted into the femoral vein, so that anesthesia could be maintained with iv urethane (1–1.4 g/kg initially, followed by supplemental doses of 0.05 g/kg as required). The depth of anesthesia was maintained to ensure the absence of corneal and pedal reflexes. The femoral artery was cannulated for monitoring arterial blood pressure. Mean arterial pressure and heart rate were calculated from the arterial pressure pulse using LabChart (ADInstruments, Bella Vista, New South Wales, Australia).

SNA recording

Lumbar SNA

After a midline abdominal incision, the left lumbar postganglionic sympathetic nerve trunk was identified and dissected free of surrounding tissue. With the aid of an operating microscope, the nerve was placed onto the bared tips of two Tef-
ion-coated silver wire electrodes, and the nerve-electrode junction was insulated electrically from surrounding tissue with a sealant (Kwik-Cast Sealant; World Precision Instruments, Sarasota, FL).

**BAT SNA activity**

Intercapular BAT was exposed through an incision in the nape of the neck. The fat pad was divided along the midline and reflected laterally. The postganglionic sympathetic nerve to the right intercapular BAT was identified and dissected free of surrounding tissue under mineral oil and was placed onto the bare tips of two Teflon-coated silver wire electrodes.

The SNA of either the lumbar or the BAT nerve was amplified using a low-noise differential amplifier (models ENG 187B and 133; Baker IDI Institute, Melbourne, Victoria, Australia), filtered (band pass 100-1000 Hz), rectified, and integrated (at 2.5-sec intervals for the BAT SNA and 0.5-sec intervals for lumbar SNA). The signal was recorded using a PowerLab data acquisition system (ADInstruments).

**BAT temperature and body core temperature**

Temperature within the intercapular BAT was recorded by placing the tip of a thermistor probe into the intercapular BAT (Fluke 73III; Fluke Australia, New South Wales, Australia). The probe was calibrated in a water bath using a mercury thermometer. Body core temperature was measured by a thermometer placed in the rectum (Fluke 52II thermometer; Fluke, Castle Hill, Australia).

**Microinjections into the lateral brain ventricle [intracerebroventricular (icv)]**

Each animal was placed prone, and the head was mounted in a Stoelting stereotaxic frame, such that bregma and lambda were positioned on the same horizontal plane. For exposure of the dorsal surface of the brain, a hole (4 mm diameter) centered 0.7 mm caudal and 1.8 mm lateral from bregma, was drilled into the skull. After the drilling procedure, the hole was covered with cotton wool soaked in normal saline to prevent drying of the exposed surface. Resistin (7 μg in 7 μl) or artificial cerebrospinal fluid (7 μl) was injected unilaterally using a fine glass micropipette (50–70 μm tip diameter) inserted into the lateral brain ventricle (stereotaxic coordinates were 0.7 mm caudal and 1.8 mm lateral from bregma, was drilled into the skull. After the drilling procedure, the hole was covered with cotton wool soaked in normal saline to prevent drying of the exposed surface. Resistin (7 μg in 7 μl) or artificial cerebrospinal fluid (7 μl) was injected unilaterally using a fine glass micropipette (50–70 μm tip diameter) inserted into the lateral brain ventricle (stereotaxic coordinates were 0.7 mm caudal to bregma, 1.8 mm lateral to midline, and 3.7 mm ventral to the surface of the dura). After the microinjection, the micropipette was left in place for 1 min. At the end of the experiment, a small amount of pontamine sky blue was microinjected using the same coordinates to confirm microinjection into the lateral ventricle.

Recombinant rat resistin was purchased from Sapphire Biosciences (lot L16251/A). Artificial cerebrospinal fluid contained 124 mM NaCl, 3.0 mM KCl, 1.3 mM NaH2PO4, 2.0 mM MgCl2, 6 mM H2O2, 26 mM NaHCO3, 10 mM glucose, and 2.0 mM CaCl2 in Milli-Q water, buffered with carbogen.

**Immunohistochemistry for Fos protein**

On completion of the icv administration of resistin/vehicle and lumbar SNA recording, the animals were euthanized with an overdose of pentobarbital (300 mg/kg, iv) and were decapitated. The brains were removed and immediately immersed in freshly prepared, ice-cold 4% paraformaldehyde in PBS (0.1 M, pH 7.2), and stored for 4 h at 4 °C. The brains were then transferred to a solution containing 20% sucrose in PBS. Serial coronal sections (40 μm thick) of the brain were cut using a cryostat (Leica, CM1900). One in five sections was collected, placed onto gelatin-coated slides, dried for 2 h at room temperature, and then processed immunohistochemically to detect Fos protein using standard immunohistochemical procedures. In brief, endogenous peroxidase activity was destroyed by incubating with 0.5% H2O2 for 30 min. The sections were then incubated in 10% normal goat serum for 60 min before 0.5% Triton X-100 (10 min) to facilitate antibody penetration. The sections were incubated in anti-Fos primary antibody [rabbit polyclonal IgG, c-Fos (K-23): sc-253; Santa Cruz Biotechnology, CA; dilution: 1:400] for 24 h.

The sections were incubated for 1 h with 1) biotinylated secondary antibody (antirabbit raised in goat, B8895; Sigma-Aldrich, Australia) and subsequently 2) Extravidin (1:400; Sigma-Aldrich, Sydney, Australia) both at room temperature.

Then the sections were incubated in 0.05% 3,3′-diaminobenzidine hydrochloride (Sigma-Aldrich, Australia) in Tris buffer (0.05 M, pH 7.6) for 10 min. The reaction was initiated by adding 5 μl of 17.5% hydrogen peroxide and terminated by washes with fresh Tris buffer. Finally, the sections were allowed to dry and were coverslipped with Depex.

**FIG. 2.** A. Screen capture of the raw recordings of lumbar SNA (LSNA) and integrated lumbar SNA (ILSNA) before and after resistin (7 μg) or vehicle (artificial cerebrospinal fluid) administered into the lateral brain ventricle. L, horizontal bar = 2 sec; vertical bar = 100 mV (lumbar SNA) and 10 mV·sec (integrated lumbar SNA). B, The percent changes in lumbar SNA from resting levels over 4 h after administration of resistin (7 μg; n = 6) or control (artificial cerebrospinal fluid; n = 7). *P < 0.05; F(1,176) = 11.85, resistin vs. control.
Fos-positive nuclei were visualized using bright-field illumination and counted at ×200 magnification.

Experimental protocols

Intracerebroventricular administration

In one series of experiments, the mean arterial pressure, heart rate, body core temperature, lumbar SNA, and BAT temperature were recorded. In these experiments, the animals were placed on a constant temperature heating pad, but no attempt was made to maintain body core temperature. In a separate series of experiments, mean arterial pressure, heart rate, body core temperature, and BAT SNA were measured. In these experiments, the animals were placed on a heating pad and the body core temperature was maintained constant by altering the temperature of the heating pad. At normal body core temperature, BAT SNA activity is virtually nonexistent. In the present work, we lowered the resting body core temperature to approximately 35°C where it was maintained. At this body core temperature, resting BAT SNA was clearly observable.

Intravenous administration

In three urethane-anesthetized rats, resistin was administered iv via a femoral vein. Mean arterial pressure, heart rate, BAT temperature, and lumbar SNA were recorded for 4 h.

In all experiments, resting levels were recorded for at least 10 min before the injection of resistin (7 μg, n = 5–8 per group for icv; n = 3 for iv) or vehicle (artificial cerebrospinal fluid, n = 4–8 per group for icv). After the injections, all variables were monitored continuously and recorded every 15 min over the next 4 h. Due to technical difficulties, in each experimental series, some variables could not be recorded in every animal.

Statistical analysis

The resting levels of mean arterial pressure, heart rate, body core temperature, and BAT temperature before the injections were compared between the resistin-treated and vehicle-injected (control) groups using Student’s unpaired t test. The integrated SNA was calculated over a period of 1–2 min at each time point and expressed as a percentage of the resting level before the injections. The resting mean arterial pressure and heart rate data in the control groups from the two experimental series have been combined because no differences were observed between the experimental series in these variables. Similarly, the mean arterial pressure and heart rate data from the resistin-treated groups have been combined. Changes in mean arterial pressure, heart rate, body core temperature, BAT temperature, lumbar SNA, and BAT SNA were compared between groups in each experimental series by using two-way ANOVA with repeated measures. All results are expressed as means ± SE. P < 0.05 was considered to be statistically significant.

Quantification of Fos-positive nuclei

Fos-positive cell nuclei were counted unilaterally in three sections containing the paraventricular nucleus (anterior, mid, and caudal levels), two sections containing the supraoptic nucleus (at the anterior and mid levels of the paraventricular nucleus), two sections containing the arcuate nucleus (located within 0.5 mm caudal to the paraventricular nucleus), and one section containing the subfornical organ. The overall mean number of Fos-positive nuclei in each area were calculated and compared between the resistin-treated (n = 6) and the control group (n = 6) using Student’s unpaired t test.

Results

Cardiovascular effects of resistin

Effect of resistin on mean arterial pressure and heart rate

The resting levels of mean arterial pressure and heart rate before the icv administration of vehicle and resistin are shown in the bar graphs in Fig. 1. There were no significant differences in the resting levels of mean arterial pressure or heart rate between the two groups (Fig. 1).

After the administration of resistin, mean arterial pressure fell by approximately 5 mmHg. Blood pressure ap-
peared to fall a similar amount in the control group but was variable. This effect was not statistically significant, and its physiological relevance is questionable. Most importantly, there was no difference between control and resistin-treated animals (Fig. 1).

After the icv injection of resistin, the effect on heart rate was quite variable over the 4-h observation period (Fig. 1). After vehicle administration, heart rate was not markedly affected. There was no significant difference in the heart rate responses between the two groups (Fig. 1).

**Effect of resistin on lumbar SNA**

Original recordings of the lumbar SNA from representative animals treated with icv resistin or vehicle are shown in Fig. 2A. The icv resistin increased lumbar SNA gradually over the observation period and reached a maximum increase of 37 ± 5% (Fig. 2B). This response was significantly different compared with the vehicle-treated group (Fig. 2).

**Effects of resistin on thermogenesis**

**Effects of resistin on BAT temperature and body core temperature**

Resting BAT temperatures before icv resistin or vehicle treatments were not significantly different between the two groups. Similarly resting body core temperatures before resistin or vehicle were not significantly different (Fig. 3).

The icv administration of resistin induced a marked reduction in BAT temperature, and this was significantly different from the vehicle-treated group (Fig. 3). The icv administration of resistin also elicited a gradual steady fall in body core temperature (Fig. 3). This was not observed in the vehicle-treated group (Fig. 3), although the difference between the groups did not attain statistical significance.

**Effects of resistin on BAT SNA**

Original recordings of BAT SNA from animals treated with icv resistin or vehicle are shown in Fig. 4A. The icv resistin reduced BAT SNA by over 50%. This occurred within 2 h of the injection of resistin, and BAT SNA remained reduced for the duration of the observation (Fig. 4B). This response was significantly different from the vehicle-treated group in which BAT SNA was slightly elevated over time (Fig. 4B). In these experiments, the body core temperature was carefully maintained constant.

**Effects of iv resistin**

To determine whether leakage of resistin from the cerebral ventricles into the systemic circulation could account for the changes described, we injected resistin iv at the same dose (7 μg) as that administered icv. The iv resistin did not significantly change mean arterial pressure, heart rate, lumbar SNA, BAT temperature, and body core temperature compared with resting levels recorded before resistin (Fig. 5).

**Effects of resistin on Fos, a marker of increased neuronal activation**

Central administration of resistin significantly increased Fos production in most of the hypothalamic nuclei examined. In the paraventricular nucleus, Fos was detected in both the magnocellular and parvocellular subnuclei (Fig. 6). Overall, there was a 7-fold increase in the number of Fos-positive cell nuclei counted in the paraventricular nucleus after the administration of resistin compared with the vehicle treatment (Fig. 7). Similarly, in the supraoptic nucleus, resistin significantly increased Fos production by 7-fold (Figs. 6 and 7). In the subfornical organ, the numbers of Fos-positive nuclei were increased by 30-fold.
with resistin treatment (Figs. 6 and 7), and this was significantly different from the vehicle-treated group. In the arcuate nucleus, there was no significant difference in the numbers of Fos-positive cell nuclei counted in the resistin-treated group compared with vehicle (Figs. 6 and 7).

Discussion

The present study is the first to directly measure the effects of resistin administration on SNA. The key findings of the study show that icv resistin increased lumbar SNA but reduced BAT SNA. As a likely consequence of the latter, the temperature of BAT decreased, and there was a decrease in body core temperature. We did not observe any significant effects on blood pressure and heart rate.

In patients and animal models of type 2 diabetes and obesity, SNA to the skeletal muscle and kidney vascular beds is increased, and this has been suggested to contribute to the incidence of cardiovascular disease in patients with those conditions. Resistin has been linked to cardiovascular disease, and there is emerging epidemiological evidence showing that plasma resistin levels are associated with the development and severity of heart failure (14, 15, 21–23). A correlation between plasma resistin levels and hypertension has also been highlighted (16, 17). Thus, resistin may contribute to cardiovascular complications, but the mechanisms involved are unknown. In the present study, we found that acute icv administration of resistin induced a significant increase in lumbar SNA. Because muscle SNA is elevated in obesity and diabetes, the effect of resistin we have observed may be a potential contributing factor to the cardiovascular complications associated with resistin.

An increase in lumbar SNA has also been observed with the adipokine leptin administered into the cerebral ventricles (24). Leptin also increased SNA to other vascular beds and elicited a concomitant rise in blood pressure. We did not observe an increase in blood pressure, suggesting there may not be a generalized increase in SNA after central administration of resistin. We investigated lumbar SNA because muscle SNA is increased in obese patients (18), a condition in which plasma resistin levels are elevated (6–9). Abdominal visceral adiposity, in particular, is closely associated with increased muscle SNA (18). Both lumbar SNA and muscle SNA are indicative of SNA to the skeletal muscle vasculature.

The present work is the first to directly measure BAT SNA after administration of resistin. We found there was a significant decrease of over 50% in BAT SNA after resistin. This indicates that resistin reduces thermogenesis, and this is supported by our finding, in separate animals, of a reduction in BAT temperature and a concomitant decrease in body core temperature. The ability of resistin to act centrally to reduce thermogenesis contrasts with the actions of leptin, which increases BAT SNA (25, 26), an action mediated via the hypothalamic arcuate nucleus (26). Thus, leptin’s actions of reducing food intake and increasing thermogenesis result in an increased energy output and resultant weight loss. By contrast, it appears that
Resistin can decrease food intake and thermogenesis, thus counteracting the reduced dietary intake with mechanisms designed for the preservation of energy. This could contribute to the lack of effect on body weight reported after resistin administration (20, 27).

There has been considerable discussion lately about the role of thermogenesis and BAT in metabolic regulation in humans. Until recently, BAT was believed to be present only in infants; however, it is now recognized that BAT is present and is metabolically active in adults (28). Stimulation of thermogenesis in BAT may have dramatic effects on energy expenditure because calculations show that activation of 40–50 g of BAT in humans could result in a 20% increase in energy expenditure (29). This could have dramatic effects on weight loss. Thus, antagonists to resistin may elicit increases in BAT SNA, which could be an efficient therapeutic mechanism to reduce body weight.

The effects observed after icv resistin were centrally mediated because iv administration of the same dose of resistin did not have marked effects. The central sites of action of resistin are unknown. Previous studies using Fos as a marker of increased neuronal activation suggested that the arcuate nucleus may be a site in which neurons are activated by resistin (20). This was only observed in fasted rats (48 h). No other hypothalamic area showed increased Fos immunoreactivity in that study (20). In mice, central administration of resistin has been found to increase Fos production in the hypothalamus, namely the arcuate, paraventricular, and dorsomedial hypothalamic nuclei (30). In our present work, we found increases in Fos immunoreactivity in the hypothalamic paraventricular, supraoptic, and subfornical nuclei but no significant increase in the arcuate nucleus and dorsomedial hypothalamus (data not shown). Our work in rats was performed in anesthetized animals, and this could complicate interpretation of the results; however, our data are consistent with the findings that activation of the paraventricular nucleus inhibits BAT SNA (31) and that the arcuate nucleus is the site at which leptin acts to increase BAT SNA (26). Species differences, whether the animals were fasted, and the duration of fasting may also account for some of the differences observed between reports. Because the receptors for resistin have not been identified as yet, unequivocal confirmation of the sites of action of resistin in the hypothalamus awaits further investigations.

In conclusion, the present findings indicate that acute administration of resistin has differential effects on SNA to tissues involved in metabolic and cardiovascular regulation. The decreased BAT SNA and the increased lumbar SNA elicited by resistin may contribute to the metabolic and cardiovascular dysfunction observed in obesity and diabetes.
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