The peptide hormone insulin is released from the pancreas and plays a pivotal role in the regulation of glucose homeostasis, body weight, and autonomic function. These effects depend, at least in part, on the ability of insulin to act within the central nervous system to inhibit food intake and sympathetic nerve activity (SNA). Strong evidence indicates that glucocorticoids impair this insulin-mediated glucose uptake and food intake. However, few data are available regarding whether glucocorticoids also modulate the sympathoexcitatory response to insulin. Therefore, the present study first confirmed that chronic administration of glucocorticoids attenuated insulin-induced increases in SNA and then investigated whether these effects were attributed to deficits in central insulin-mediated responses. Male Sprague-Dawley rats were given access to water or a drinking solution of the glucocorticoid agonist dexamethasone (0.3 µg/ml) for 7 days. A hyperinsulinemic-euglycemic clamp significantly increased lumbar SNA in control rats. This response was significantly attenuated in rats given access to dexamethasone for 7, but not 1, days. Similarly, injection of insulin into the lateral ventricle or locally within the arcuate nucleus (ARC) significantly increased lumbar SNA in control rats but this response was absent in rats given access to dexamethasone. The lack of a sympathetic response to insulin cannot be attributed to a generalized depression of sympathetic function or inactivation of ARC neurons as electrical activation of sciatic afferents or ARC injection of gabazine, respectively, produced similar increases in SNA between control and dexamethasone-treated rats. Western blot analysis indicates insulin produced similar activation of Akt Ser473 and pS6 Ser240/244 in the ventral hypothalamus of control and dexamethasone-treated rats. Collectively, these findings suggest that dexamethasone attenuates the sympathoexcitatory actions of insulin through a disruption of ARC neuronal function downstream of Akt or mammalian target of rapamycin (mTOR) signaling.

Glucocorticoids attenuate the central sympathoexcitatory actions of insulin. J Neurophysiol 112: 2597–2604, 2014. First published September 3, 2014; doi:10.1152/jn.00514.2014.—Insulin acts within the central nervous system to regulate food intake and sympathetic nerve activity (SNA). Strong evidence indicates that glucocorticoids impair insulin-mediated glucose uptake and food intake. However, few data are available regarding whether glucocorticoids also modulate the sympathoexcitatory response to insulin. Therefore, the present study first confirmed that chronic administration of glucocorticoids attenuated insulin-induced increases in SNA and then investigated whether these effects were attributed to deficits in central insulin-mediated responses. Male Sprague-Dawley rats were given access to water or a drinking solution of the glucocorticoid agonist dexamethasone (0.3 µg/ml) for 7 days. A hyperinsulinemic-euglycemic clamp significantly increased lumbar SNA in control rats. This response was significantly attenuated in rats given access to dexamethasone for 7, but not 1, days. Similarly, injection of insulin into the lateral ventricle or locally within the arcuate nucleus (ARC) significantly increased lumbar SNA in control rats but this response was absent in rats given access to dexamethasone. The lack of a sympathetic response to insulin cannot be attributed to a generalized depression of sympathetic function or inactivation of ARC neurons as electrical activation of sciatic afferents or ARC injection of gabazine, respectively, produced similar increases in SNA between control and dexamethasone-treated rats. Western blot analysis indicates insulin produced similar activation of Akt Ser473 and pS6 Ser240/244 in the ventral hypothalamus of control and dexamethasone-treated rats. Collectively, these findings suggest that dexamethasone attenuates the sympathoexcitatory actions of insulin through a disruption of ARC neuronal function downstream of Akt or mammalian target of rapamycin (mTOR) signaling.

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sone eliminated the increase in muscle SNA to a hyperinsulinemic-euglycemic clamp in humans (Scherrer et al. 1993). Therefore, the initial purpose of this study was to confirm that glucocorticoid administration attenuated the sympahtoexcitatory response to a hyperinsulinemic-euglycemic clamp in rodents. Although a previous report indicates that dexamethasone interferes with insulin-mediated transport across the blood-brain-barrier (Baura et al. 1996), subsequent experiments were designed to test the hypothesis that dexamethasone attenuates the centrally mediated insulin responses by icv injection of insulin or local injection of insulin into the ARC nucleus. A final set of experiments examined whether dexamethasone attenuated insulin-stimulated increases in Akt Ser473 and rpS6 Ser240/244 of the ventral hypothalamus since prior evidence suggests that the sympahtoexcitatory response to insulin depends on PI3K-Akt signaling in the hypothalamus (Rahmouni et al. 2004) and observations in other tissues suggest that glucocorticoids interfere with insulin-mediated actions through a disruption in PI3K-Akt and mTORC1 signaling.

MATERIALS AND METHODS

Animals. All of the experimental procedures conform to the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at the Pennsylvania State College of Medicine. Male Sprague-Dawley rats (initial body weight: 275–325 g; Charles River Laboratories) were singly housed in a temperature-controlled room (22–23°C) with a 12:12-h light-dark cycle. Rats were fed standard chow (Harlan Teklad 2018) and given access to deionized water or a dexamethasone solution (0.3 μg/ml) for either 1 or 7 days. The dexamethasone solution was replaced daily and diluted from a stock solution (2 mg/ml; Bimeda). Food and water intake were measured daily. All experiments were conducted in the ab libitum fed state.

Sympathetic nerve recordings. Animals were anesthetized with isoflurane (2–5% in 100% O₂) and instrumented with a femoral arterial catheter for arterial blood pressure (ABP) measurement, a double-lumen catheter in the femoral vein for intravenous infusions, and a brachial arterial catheter for blood sampling. The lumbar and renal sympathetic nerves were isolated through a ventral midline and a retroperitoneal incision as described previously (Bardgett et al. 2010; Luckett et al. 2013). Once variables returned to baseline values for 20 min, gabazine (1 mM, 20 nl) was injected unilaterally into the arcuate nucleus using single-barrel micropipettes (20 μM OD) as described previously (Bardgett et al. 2010; Luckett et al. 2013). Variables were recorded for an additional 2 h. Drug solutions contained 0.2% rhodamine of FITC-labeled microspheres. At the end of experiments, animals were perfused transcardially with 4% paraformaldehyde. Brains were harvested, postfixed in 4% paraformaldehyde, and embedded in paraffin for cross-sections. Every 5th section was stained for the arcuate nucleus. The area containing labeled microspheres was determined using ImageJ. To test whether dexamethasone attenuates sympathetic responses to stimuli other than insulin, the sciatic afferent nerve was isolated and stimulated electrically (1 ms, 500 μA, 5–50 Hz) at all frequencies (1, 2, 5, 10, and 20 Hz) while animals were paralyzed with gallamine triethiodide (25 mg/kg iv).

Experiment 1–icv injection of insulin. To establish that dexamethasone attenuates centrally mediated actions of insulin, SNA responses were measured in response to lateral ventricle injection of insulin. Initially, chronic brain cannulas targeted at the lateral ventricle were implanted in naïve rats as described previously (Bardgett et al. 2010; Stocker et al. 2003). Cannula placement was verified by a positive drinking response (>3 ml) to angiotensin II injection (20 ng/μl). Then, animals were given access to water or dexamethasone solution for 7 days, and prepared for SNA and ABP recordings as described above. Insulin (50 mU/μl) was injected into the lateral ventricle, and responses were measured for an additional 120 min. Blood glucose was measured every 30 min. At the end of the experiment, cannula placement was verified again by the presence of dye in the third and fourth ventricles after injection of Evan’s blue dye (1%, 2 μl) through the cannula.

Experiment 3–arcuate microinjection. A third set of experiments was performed to determine whether the central actions of insulin were attenuated by dexamethasone in the ARC nucleus. Rats were given access to water or dexamethasone for 7 days and then prepared for ABP and SNA recordings as described above. Rats were placed into a stereotaxic head frame. A small craniotomy was performed to gain access to the cortex overlying the arcuate nucleus. After variables stabilized for 20 min, gabazine (1 mM, 20 nl) was injected unilaterally into the arcuate nucleus using single-barrel micropipettes (20 μM OD) as described previously (Luckett et al. 2013). Once variables returned to baseline values for 20 min, insulin (4 μU, 30 nl) was injected bilaterally using a glass pipette lowered into each arcuate nucleus. Variables were recorded for an additional 2 h. Drug solutions contained 0.2% rhodamine of FITC-labeled microspheres. At the end of experiments, animals were perfused transcardially with 4% paraformaldehyde. Brains were harvested, postfixed in 4% paraformaldehyde, sectioned at 100 μm using a vibratome, and counterstained as described previously (Luckett et al. 2013). Data are reported for those animals that displayed labeled microspheres within the boundaries of the ARC as described previously (Luckett et al. 2013).

Experiment 4–Western blot analysis. To assess the activation of the PI3K/Akt pathway in response to insulin following glucocorticoid treatment, rats were first instrumented with chronic brain cannulas targeted at the lateral ventricle. Placement was verified by a drinking response to angiotensin II as described above. Animals were then given access to water or dexamethasone solution for 7 days and acclimated to the ventricular injection procedure each day. On day 7, rats received an injection of artificial cerebrospinal fluid (aCSF; 2 μl) or insulin (50 mU/2 μl). At 30 min after injection, rats were decapitated and a block of the ventral hypothalamus was isolated under stereomicroscope and immediately frozen in liquid nitrogen. The block of tissue was defined laterally by the fornix, dorsally by the fornix, rostrally by the optic chiasm, and caudally by mammillary bodies. Samples were stored at −80°C.

Hypothalamic samples (1.5–13 mg) were homogenized using a glass mortar and pestle in 7 vol of ice-cold buffer consisting of the following: 50 mM HEPES pH 7.8, 137 mM NaCl, 2 mM EDTA, 1 mM magnesium chloride, 1 mM sodium orthovanadate, 1% NP-40, and 1 Complete Mini EDTA-free Protease Inhibitor Cocktail tablet...
Values are means ± SE. Numbers in parentheses represents the number of animals in the respective group for the cardiovascular measurements. ABP, arterial blood pressure; SNA, sympathetic nerve activity. *P < 0.05 vs. control group.

Chronic dexamethasone treatment attenuates sympathoexcitatory response to a hyperinsulinemic-euglycemic clamp. To confirm a previous study in humans (Scherrer et al. 1993) that dexamethasone attenuates the sympathoexcitatory response to insulin, rats were given access to water or dexamethasone solution for 1 or 7 days and then infused with insulin iv under euglycemic conditions. As previously reported (Bardgett et al. 2010; Luckett et al. 2013; Ward et al. 2011), a hyperinsulinemic-euglycemic clamp significantly increased lumbar SNA but did not alter mean ABP in control rats (Fig. 1). In contrast, the

**Fig. 1. Example of arterial blood pressure (ABP), mean ABP (grey line), integrated lumbar sympathetic nerve activity (SNA), and 1-s segments of raw lumbar SNA of rats drinking water (A) or dexamethasone (B; Dex) for 7 days and then infused with insulin plus dextrose.**
lumbar sympathoexcitatory response was significantly attenuated in rats drinking the dexamethasone solution for 7 days but not after 1 day (Fig. 1). Despite significantly higher plasma insulin levels in rats drinking dexamethasone for 7 days, the glucose infusion rate to maintain euglycemia was significantly less in rats drinking dexamethasone for 7 days vs. control rats or rats drinking dexamethasone for 1 day. Heart rate did not change from baseline values (control: 355 ± 12 beats/min; 1 day: 375 ± 15 beats/min; 7 day: 365 ± 10 beats/min; \( P > 0.3 \) from overall ANOVA). In addition, a hyperinsulinemic-euglycemic clamp did not alter renal SNA in any group (data not shown).

To test whether dexamethasone treatment produced a non-specific attenuation of sympathetic reflexes, the sciatic nerve afferents were stimulated electrically across a range of frequencies. As illustrated in Fig. 2, electrical activation of sciatic nerve afferents produced frequency-dependent increases in mean ABP, lumbar SNA, and renal SNA within both groups. However, the responses were not statistically different between rats drinking water vs. dexamethasone.

Dexamethasone treatment attenuates sympathoexcitatory response to icv injection of insulin. To directly test whether dexamethasone attenuated the central sympathoexcitatory action of insulin independent of blood-brain-barrier transport, insulin was injected into the lateral ventricle of rats drinking either water or dexamethasone for 7 days. As previously reported (Luckett et al. 2013; Muntzel et al. 1994b; Ward et al. 2011), icv injection of insulin in control rats significantly increased lumbar SNA without a change in mean ABP (Fig. 3). In marked contrast, the sympathoexcitatory response to icv insulin in dexamethasone-treated rats was significantly less at 90 and 120 min (Fig. 3C). The icv insulin did not alter blood glucose (Fig. 3) or heart rate (control: 410 ± 14 beats/min vs. 7-day dexamethasone: 417 ± 16 beats/min).

Dexamethasone treatment attenuates sympathoexcitatory response to arcuate nucleus injection of insulin but not gabazine. As previous studies indicate that insulin acts on ARC neurons to increase SNA (Cassaglia et al. 2011; Luckett et al. 2013), a third series of experiments were performed to directly assess the extent by which dexamethasone attenuates the actions of insulin in the ARC. As expected (Cassaglia et al. 2011; Luckett et al. 2013), bilateral injection of insulin into the ARC...
of control rats significantly increased lumbar SNA but did not alter mean ABP (Fig. 4A) or renal SNA (data not shown). In marked contrast, lumbar SNA did not change after ARC injection of insulin in rats drinking dexamethasone for 7 days (Fig. 4A). Blood glucose (control: 71 ± 3 mg/dl; 7-day dexamethasone: 75 ± 2 mg/dl) and heart rate (control: 377 ± 15 beats/min; 7-day dexamethasone: 385 ± 13 beats/min) did not change after ARC injection of insulin in either group.

To test whether dexamethasone treatment produced a general inactivation of ARC neurons, the same animals received a unilateral injection of gabazine. As reported previously (Kawabe et al. 2012; Luckett et al. 2013), injection of gabazine into the ARC significantly increased lumbar SNA and mean ABP (Fig. 4B). The magnitude of these responses was not different between rats drinking water or dexamethasone for 7 days. The location of injection sites was also not different between the groups (Fig. 4C).

**Dexamethasone treatment did not interfere with central insulin signaling.** To investigate whether dexamethasone interferes with central insulin signaling, a final set of experiments analyzed the expression of several proteins in the ventral hypothalamus after the central injection of insulin. As expected, icv insulin in control rats significantly increased the expression of Akt Ser473 (Fig. 5A) and rpS6 Ser240/244 (Fig. 5B). However, icv insulin also increased the expression of Akt Ser473 (Fig. 5A) and rpS6 Ser240/244 in dexamethasone-treated rats (Fig. 5, A and B), and the levels of Akt Ser473 and rpS6 Ser240/244 after insulin treatment did not differ between control and dexamethasone-treated rats. Autophosphorylation of mTOR on residue Ser2481 was not different between treatment groups before normalization. However, levels of total mTOR protein were increased in all groups compared with control animals injected with aCSF. Therefore, when mTOR Ser2481 is expressed relative to total mTOR, significant decreases in this autophosphorylation site were detected in both control animals injected with insulin and dexamethasone-treated animals injected with aCSF (Fig. 5C).

**DISCUSSION**

Despite evidence that glucocorticoids act centrally to modulate glucose homeostasis and food intake (Mori et al. 2009;
Norman et al. 2004; Shimizu et al. 2010; Yi et al. 2012), only one prior study directly assessed whether glucocorticoids altered the sympathetic response to insulin (Scherrer et al. 1993). The initial set of experiments in this study confirmed this observation in rodents as 7, but not 1, days of dexamethasone administration attenuated the increase in lumbar SNA during a hyperinsulinemic-euglycemic clamp. Subsequent experiments were then designed to determine whether glucocorticoids act centrally to disrupt insulin-mediated responses in SNA and PI3K-Akt/mTORC1 signaling. This series of experiments provide several novel observations including: 1) dexamethasone attenuates the increase in lumbar SNA after icv injection of insulin, 2) ARC injection of insulin fails to increase lumbar SNA in dexamethasone-treated rats, 3) dexamethasone does not affect the sympathetic responses to electrical activation of sciatic afferents or ARC injection of gabazine, and 4) dexamethasone does not affect the insulin-induced activation of Akt or rpS6 in the ventral hypothalamus. Collectively, these findings suggest glucocorticoids act centrally within the ARC nucleus to attenuate the sympathoexcitatory actions of insulin through a disruption of ARC neuronal function that is independent of Akt or mTORC1 signaling.

A number of studies have documented that glucocorticoids impair insulin signaling and function (Ruzzin et al. 2005; Shah et al. 2000; Yi et al. 2012). To our knowledge, only one prior study examined whether the administration of glucocorticoids attenuates the sympathoexcitatory response to insulin (Scherrer et al. 1993). Scherrer et al. reported that 48-h administration of dexamethasone abolished the increase in muscle SNA and calf vasodilation during a hyperinsulinemic-euglycemic-clamp. In contrast, dexamethasone did not affect the sympathetic and pressor responses to a Valsalva maneuver or cold pressor test. The present findings confirm these observations as dexamethasone administration for 7 days significantly attenuated the lumbar sympathoexcitatory response to a hyperinsulinemic-euglycemic clamp. The smaller sympathetic response was independent of changes in plasma insulin as dexamethasone-treated rats had significantly higher plasma insulin levels at 60 and 120 min compared with control rats (although the change in plasma insulin concentration from baseline levels was not different between groups). Again, these findings cannot be attributed to a general depression of all sympathetic circuits or reflexes, as electrical activation of sciatic nerve afferents produced similar increases in SNA and ABP between control and dexamethasone-treated rats. Therefore, these findings confirm the earlier study of Scherrer et al. (1993) and indicate that chronic dexamethasone treatment attenuates the sympathoexcitatory actions of insulin in rodents.

Accumulating evidence indicates that glucocorticoids act centrally to impact glucose homeostasis and body weight (Mori et al. 2009; Norman et al. 2004; Shimizu et al. 2010; Yi et al. 2012). A major goal of the present study was to establish whether the smaller sympathetic response to a hyperinsulinemic-euglycemic clamp in dexamethasone-treated animals (or humans) was attributable to a disruption of centrally mediated insulin responses. In the present study, injection of insulin into the lateral ventricle increased lumbar SNA in control rats, but this effect was significantly attenuated in rats treated with dexamethasone. Additionally, direct injection of insulin into the ARC increased lumbar SNA in control but not dexamethasone-treated rats. In marked contrast, ARC injection of the GABA_A receptor antagonist gabazine produced similar increases in SNA between control and dexamethasone-treated rats. Collectively, these findings suggest that dexamethasone induces deficits in centrally mediated actions of insulin within ARC neurons. These findings are consistent with previous reports that glucocorticoids attenuate centrally mediated effects of insulin on hepatic glucose production (Yi et al. 2012). It is also noteworthy that dexamethasone has been reported to decrease insulin transport from plasma to the cerebrospinal fluid (Baura et al. 1996), and the decreased transport of insulin may contribute to the attenuated sympathetic response during a hyperinsulinemic-euglycemic clamp. The present findings demonstrate that glucocorticoids have an additional central effect to interfere with insulin-mediated responses.

Consistent with previous observations in our laboratory (Bardgett et al. 2010; Luckett et al. 2013; Ward et al. 2011), but in contrast to those of others (Morgan and Rahmouni 2010; Rahmouni et al. 2003, 2004), we did not observe an increase in renal SNA during a hyperinsulinic-euglycemic clamp or after ARC injection of insulin. The reason for the discrepancy is not clear. However, data from humans indicate that a hyperinsulinemic-euglycemic clamp increased muscle SNA but failed to alter renal norepinephrine spillover (Gudbjorssdottir et al. 1994). While it remains unclear why insulin would directly alter lumbar but not renal SNA, these data highlight the ability of the central nervous system to differentially control SNA in response to various stimuli.

The actions of insulin are mediated by PI3K/Akt associated activation of mTORC1 signaling in both peripheral tissues as well as the central nervous system (Inoki et al. 2002; Rahmouni et al. 2004; Sancak et al. 2007). Since previous reports in skeletal muscle indicate that glucocorticoids impair these same insulin-signaling pathways (Ruzzin et al. 2005; Shah et al. 2000), we hypothesized that the smaller sympathetic response to insulin would be attributed to a blunted activation of the PI3K/Akt and mTORC1 pathways. Instead, we found that phosphorylation of Akt, a protein downstream of PI3K, and rpS6, a protein phosphorylated by one of the mTORC1 primary substrates, p70S6K1, were unchanged by dexamethasone treatment under both basal and insulin-stimulated conditions. While these data may suggest that the sympathetic response to insulin does not depend on PI3K/Akt and mTORC1 pathways, Rahmouni et al. (2004) have reported that centrally administered insulin increases PI3K/Akt in the ventral hypothalamus and icv pretreatment with LY294002 or wortmannin prevented the insulin-induced increase in lumbar SNA. Therefore, a plausible explanation is that dexamethasone disrupts insulin signaling downstream of PI3K/Akt or mTORC1. Since tissue punches were collected from the ventral hypothalamus, it is possible that changes specifically within the ARC or within subsets of ARC neuronal populations [neuropeptide Y (NPY) vs. proopiomelanocortin] were missed.

The lack of basal changes following dexamethasone treatment contrasts ex vivo work in rat hypothalamic organotypic cultures in which incubation with dexamethasone for 24-h significantly decreased phosphorylation of both p70S6K1 and rpS6 (Shimizu et al. 2010). A possible explanation for these differences, aside from the obvious methodological variations, is the influence of the animals’ nutritional status (i.e., fed) and/or time of tissue collection following injection of insulin on PI3K/Akt/mTORC1 signaling. Previous work has shown
maximal mechanisms for these effects may be tissue or cell
coids have profound and widespread effects on metabolic
activate this pathway. These findings indicate that glucocorti-
signaling, central insulin administration was found to robustly
association with a blunted activation of PI3K/Akt or mTORC1
was not a necessary event in the activation of S6K1 and 4E-BP1 and is in agreement with the current findings
(Peterson et al. 2000; Soliman et al. 2010). Evidently, the
effects of short-term dexamethasone treatment on insulin-
signaling pathways are differentially regulated in peripheral
and central tissues while insulin-induced-signaling pathways
within the central nervous system appear to be somewhat
resistant to prolonged exposure to dexamethasone in contrast to
the effects observed on SNA.

An alternative explanation for the findings is that glucocor-
ticoids may attenuate the sympathoexcitatory response to in-
sulin through activation of NPY-containing neurons in the
ARC. First, glucocorticoid administration upregulates the
expression of NPY in the ARC (Akabayashi et al. 1994; Yi et al.
2012). Second, NPY-positive ARC neurons densely innervate
PVN (Broberger et al. 1999), and injection of NPY into the
PVN decreases SNA and ABP (Cassaglia et al. 2014). In
agreement, sympathetic and cardiovascular responses evoked from
the ARC are partially mediated by NPY receptor activation
in the PVN (Kawabe et al. 2012). Moreover, a recent study
reported that local infusion of dexamethasone into the ARC
attenuated insulin-mediated suppression of glucose production
(Yi et al. 2012) and this effect was prevented by icv antago-
nist of NPY receptors (Yi et al. 2012). This evidence suggests
that glucocorticoids may attenuate the sympathoexcitatory ac-
tions of insulin through the upregulation of NPY signaling;
however, this hypothesis has not been tested.

In summary, the present study demonstrates that glucocor-
ticoids attenuate the central sympathoexcitatory actions of
insulin. Although we originally hypothesized that an attenua-
tion of the sympathetic response by glucocorticoids would be
associated with a blunted activation of PI3K/Akt or mTORC1
signaling, central insulin administration was found to robustly
activate this pathway. These findings indicate that glucocorti-
coids have profound and widespread effects on metabolic
function in both peripheral and central tissues, but the under-
lying mechanisms for these effects may be tissue or cell
specific. Future studies will be needed to understand the
cellular mechanisms within ARC and perhaps NPY neurons
mediated by glucocorticoids.

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AUTHOR CONTRIBUTIONS
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ments; J.L.S., M.E.B., L.W., C.H.L., and S.D.S. analyzed data; J.L.S., M.E.B.,
L.W., C.H.L., and S.D.S. interpreted results of experiments; J.L.S. and S.D.S.
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REFERENCES
Akabayashi A, Watanabe Y, Wahlestedt C, McEwen BS, Paez X, Leibowitz SF. Hypothalamic neuropeptide Y, its gene expression and receptor
activity: relation to circulating corticosterone in adrenalectomized rats.
Anderson EA, Hoffman RP, Balon TW, Sinkey CA, Mark AL. Hyperinsu-
linemia produces both sympathetic neural activation and vasodilation in
Aronsson M, Fuxe K, Dong Y, Agnati LF, Okret S, Gustafsson JA.
Localization of glucocorticoid receptor mRNA in the male rat brain by in
Bardgett ME, McCarthy JJ, Stocker SD. Glutamatergic receptor activation
in the rostral ventrolateral medulla mediates the sympathoexcitatory re-
Baura GD, Foster DM, Kiyaiya K, Porte D Jr, Kahn SE, Schwartz MW.
Insulin transport from plasma into the central nervous system is inhibited
Begg DP, Woods SC. The central insulin system and energy balance. Handb
Broberger C, Visser TJ, Kuhar MJ, Hokfelt T. Neuropeptide Y innervation and
neuropeptide-Y-Y1-receptor-expressing neurons in the paraventricular
hypothalamic nucleus of the mouse. Neuroendocrinology 70: 295–305,
1999.
Cassaglia PA, Hermes SM, Aicher SA, Brooks VL. Insulin acts in the
arcuate nucleus to increase lumbar sympathetic nerve activity and baroreflex
Cassaglia PA, Shi Z, Li B, Reis WL, Clute-Reinig NM, Stier JE, Brooks
VL. Neuropeptide Y acts in the paraventricular nucleus to suppress symp-
1675, 2014.
Geer EB, Islam J, Buettner C. Mechanisms of glucocorticoid-induced insulin
resistance: focus on adipose tissue function and lipid metabolism. Endocri-
The effect of metformin and insulin on sympathetic nerve activity, norepi-
 nephrine spillover and blood pressure in obese, insulin resistant, normogly-
Harlan SM, Guo DF, Morgan DA, Fernandes-Santos C, Rahmouni K.
Hypothalamic mTORC1 signaling controls sympathetic nerve activity and
Hill JW, Williams KW, Ye C, Luo J, Balthasar N, Coppari R, Cowley MA,
Canley LC, Lowell BB, Elmqquist JK. Acute effects of leptin require PI3K
signaling in hypothalamic proopiomelanocortin neurons in mice. J Clin
Inoki K, Li Y, Zhu T, Wu J, Guan KL. TSC2 is phosphorylated and
inhibited by Akt and suppresses mTOR signaling. Nat Cell Biol 4: 648–
657, 2002.
Kalsbeek A, Bruinstroep E, Yi CX, Klieverik LP, La Fleur SE, Flies E.
Hypothalamic control of energy metabolism via the autonomic nervous


