Hypothalamic adrenergic receptor changes in the metabolic syndrome of genetically obese (ob/ob) mice

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Boundy, Virginia A., and Anthony H. Cincotta. Hypothalamic adrenergic receptor changes in the metabolic syndrome of genetically obese (ob/ob) mice. Am J Physiol Regulatory Integrative Comp Physiol 279: R505–R514, 2000.—The genetically, seasonally, and diet-induced obese, glucose-intolerant states in rodents, including ob/ob mice, have each been associated with elevated hypothalamic levels of norepinephrine (NE). With the use of quantitative autoradiography on brain slices of 6-wk-old ob/ob (ob/ob) and lean mice, the adrenergic receptor populations in several hypothalamic nuclei were examined. The binding of [125I]iodocyanopindolol to β2- and βα-adrenergic receptors in ob/ob mice was significantly increased in the paraventricular hypothalamic nucleus (PVN) by 30 and 38%, in the ventromedial hypothalamus (VMH) by 23 and 72%, and in the lateral hypothalamus (LH) by 10 and 15%, respectively, relative to lean controls. The binding of [125I]iodo-4-hydroxyphenyl-ethyl-aminomethyl-tetralone to αα-adrenergic receptors was also significantly increased in the PVN (26%), VMH (67%), and LH (21%) of ob/ob mice. In contrast, the binding of [125I]paraiodoclonidine to αα-adrenergic receptors in ob/ob mice was significantly decreased in the VMH (38%) and the dorsomedial hypothalamus (17%) relative to lean controls. This decrease was evident in the α2A- but not the α2βC-receptor subtype. Scatchard analysis confirmed this decreased density of α2-receptors in ob/ob mice. Together with earlier studies, these changes in hypothalamic adrenergic receptors support a role for increased hypothalamic NE activity in the development of the metabolic syndrome of ob/ob mice.

EVIDENCE FROM SEVERAL LABORATORIES implicates an important role for ventromedial hypothalamic noradrenergic activity in the regulation of peripheral glucose and lipid metabolism. Acute administration of norepinephrine (NE) into the ventromedial hypothalamic nucleus (VMH) induces rapid and concurrent increases in plasma glucose, free fatty acids (FFA), insulin, glucagon, and sympathetic nervous system (SNS) activity (16, 22, 56-58, 62). This induced neuroendocrine profile is a unique characteristic feature of the obese diabetic condition (15). Indeed, chronic infusion of NE into the VMH of normal animals does induce the obes, hyperinsulinemic, glucose-intolerant state (metabolic syndrome) without producing chronic hyperphagia (13, 40, 42, 59). Importantly, the endogenous hypothalamic (particularly VMH) levels of NE and/or its metabolites have been reported to be elevated in a wide variety of obese, glucose-intolerant animal models, including the ob/ob mouse (20, 28, 43, 44, 47). Moreover, the elevated hypothalamic NE levels in ob/ob mice do not appear to be a secondary consequence of the obesity (49), in agreement with the abovementioned responses to exogenous NE. Furthermore, the electrophysiological responsiveness to NE within the VMH is markedly enhanced in obese-hyperglycemic (ob/ob) vs. lean-euglycemic mice (30). Collectively, these electrophysiological and neuropharmacological results suggest that in the obese-hyperglycemic state of ob/ob mice, VMH NE levels as well as the VMH response to NE are paradoxically both elevated. As such, information relating to the NE receptor profile within the VMH of obese-hyperglycemic vs. lean-euglycemic animals is vital to the understanding of the neurophysiology of VMH NE regulation of peripheral glucose and lipid metabolism. To date, however, no systematic characterization of noradrenergic receptor subtypes within discrete hypothalamic nuclei, such as the VMH, of ob/ob vs. lean mice has been undertaken. General binding characteristics of nonspecific noradrenergic ligands to whole hypothalamus, however, have been reported (48). We therefore examined differences in the noradrenergic receptor profile (including α1-, total αα-, α2A-, α2βC-, β1-, and β2-ligand binding characteristics) of obese-hyperglycemic (ob/ob) and lean-euglycemic (+/+?) mice within the VMH and other hypothalamic nuclei involved in the regulation of peripheral metabolism.

MATERIALS AND METHODS

Animals. Four-week-old female genetically obese (ob/ob); body wt = 30–38 g) and lean (C57BL/6J +/??; body wt = 17–21 g) mice (Jackson Laboratory, Bar Harbor, ME) were group housed under 12:12-h light-dark daily photocycles with food and drink ad libitum. Animals were killed at 4 h after light onset (HALO) 2 wk after acclimation to the animal care facility at Ergo Science. The ob/ob mice at this age and weight are very hyperglycemic and are rapidly accruing body adiposity relative to lean litter mates (54).

Tissue preparation. Mice brains were removed rapidly after decapitation, blocked, frozen on dry ice, and stored at −80°C. Brains were then embedded in Tissue-Tek OCT com-

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compound (VWR; Rochester, NY) and returned to −80°C. Serial sections (20 μm) were obtained on a cryostat, at −14 to −16°C, thaw-mounted onto chrom-alum-coated glass slides, and stored at −20°C until receptor autoradiography was performed. The experimental protocol was reviewed and approved by the Animal Care Committee at Ergo Science.

**Autoradiography.** Slide-mounted sections were removed from −20°C and allowed to equilibrate to room temperature. After preincubation in buffer for 20 min, sections were incubated at room temperature in a moist chamber with ~100 μlsection of ligand in buffer. Within each hypothalamic area of interest, receptor ligand binding assays for each noradrenergic receptor type (or subtype) were conducted in duplicate on 20-μm sections taken from serial coronal sections at 120-μm distances from each other, through the length of the nucleus. Adjacent sections were incubated concurrently under appropriate conditions for nonspecific and/or subtype-specific binding. Sections from lean and obese animals were incubated simultaneously in the same incubation chambers. For β-receptors, binding conditions were modified from previously published reports (7, 23, 46, 52). Sections were incubated for 150 min in buffer of 50 mM Tris, pH 7.4, 154 mM NaCl containing 20 pM [125I]iodocyanopindolol ([125I]CYP; Amersham) and 3 μM serotonin (RBI, Natick, MA) to block binding to 5-hydroxytryptamine 1B receptors. Nonspecific binding was defined in the presence of 2 μM propranolol (RBI). β2-Specific binding was performed in the presence of 70 nM IC118551 (RBI), a β2-adrenergic receptor antagonist. β2-Specific binding was performed in the presence of 10 nM CGP20712A (RBI), a β3-adrenergic receptor antagonist. For α2-receptors, sections were incubated for 90 min in buffer of 170 mM Tris, pH 7.4, and 20 mM MgCl2 containing 550 pM or 50–2,200 pM [125I]paraiodoclonidine ([125I]PIC; NEN DuPont, Boston, MA) as described previously (1). Nonspecific binding was defined in the presence of 10 μM phenolamine (RBI). Specific ligand binding to α2A-receptors was performed in the presence of 400 nM prazosin (RBI), an α2HCR-adrenergic receptor antagonist (10, 21). Specific α2mHC-subtype binding was performed in the presence of 20 nM oxymetazoline (RBI), an α2A-adrenergic receptor partial agonist (21, 64). For analysis of α2-receptor ligand binding, sections were incubated for 120 min in buffer of 50 mM Tris, pH 7.4, and 1 mM EDTA containing 50 pM [125I]iodo-4-hydroxyphenyl-ethyl-amino-methyl-tetralone ([125I]HEAT, NEN DuPont) as described previously (29). Nonspecific binding was defined in the presence of 10 μM phenolamine (RBI). For all receptors, slides were rapidly rinsed in ice-cold buffer to remove excess ligand, washed twice in ice-cold buffer, and rinsed in ice-cold distilled water to remove buffer salts. Sections were allowed to dry and then exposed to [H]-Hyperfilm (Amersham, Piscataway, NJ) for 1–2 days. After exposure to film, sections representing nonspecific binding were stained with cresyl violet to identify the location of the hypothalamic nuclei of interest. Areas were chosen because of their known importance in the regulation of peripheral glucose and lipid metabolism. These included the paraventricular hypothalamic nucleus (PVN), the anterior (AH) and lateral (LH) areas of the hypothalamus, the VMH, and the dorsomedial nucleus of the hypothalamus (DMH). Because of the small size of the hypothalamic areas analyzed, not all receptor binding studies could be performed on a single group of animals. Separate groups of lean and ob/ob mice (n = 5–8/genotype/group; as in legends to Figs. 1–6) were used for 1) β1-, β2-, and α2A-subtype binding; 2) α1A- and α2ATotal, α2H, and α2H-subtype binding; and 3) Scatchard plot analysis of α2A-subtype binding.

**Image analysis.** Images were digitized from film using a charge-coupled device camera and Scion Image. Areas of interest were identified on the cresyl violet-stained nonspecific sections. By overlaying the nonspecific digital image with the adjacent total binding image, the density (pixels) of binding could be quantified from the appropriate area of interest. The binding density of the area identified by the cresyl violet stain was quantified from both total and nonspecific sections. Specific binding was determined by subtracting the nonspecific density value from the total density value. The Scion Image system was calibrated to disintegrations per minute per milligram protein using autoradiographic [125I]microscales (Amersham) exposed on each piece of film as follows. Brain sections used for radiolabeled ligand binding and [125I]microscale standards were both simultaneously exposed on every autoradiographic film. Autoradiographic [125I]microscales consist of 10 layers of radioactive colorless polymer arranged in order of increasing specific activity separated by colored nonradioactive layers. The [125I] is uniformly incorporated into each polymer layer at the molecular level to allow accurate quantitation of [125I]-labeled compounds in a wide variety of samples. The thickness of the microscale was 20 μm, the same as that of the tissue samples. The units for each [125I]microscale standard are disintegrations per minute per milligram of polymer and the standards range from 1.25 to 160 nCi/mg of polymer. The Amersham product specifications state that the brain tissue equivalent in milligrams protein is 47% of the polymer value. Therefore, tissue equivalents in milligrams protein were calculated as 47% of the polymer value (adjusted for decay to the day of exposure). Autoradiographic data in pixels per area were then converted to disintegrations per minute per milligram protein by simply generating a standard curve on each piece of film with tissue sections. Density values were obtained for the standards, and a standard curve was generated using the tissue equivalent values in disintegrations per minute per milligram protein. The Scion Image program was then calibrated according to this curve so that all future density measurements taken from that piece of film were reported in both pixels per area and disintegrations per minute per milligram protein. Results are also reported as the percent change of binding seen in lean animals under identical binding conditions. This allows the results from multiple experiments to be compared more easily.

**Statistics.** Specific binding for all ligands was determined for multiple brain regions by subtracting the nonspecific binding, measured in the presence of excess unlabeled competing ligand, from the total binding. Maximal binding and dissociation constant (Bmax and Kd, respectively) values were determined by Scatchard transformation of saturation binding data. All values are expressed as group means ± SD. Radioligand binding to receptor sites in discrete hypothalamic nuclei of ob/ob vs. lean mice was analyzed by a two-way ANOVA followed by t-tests for direct between-group comparisons of ligand binding in specific nuclei. Intergroup differences in Scatchard Bmax and Kd values were analyzed by t-test for unpaired samples.

**RESULTS**

There was a significant interaction of genotype and hypothalamic area on ligand binding for α1A- and/ or β1- and β2-ligands (2-way ANOVAs; P < 0.05). Major differences between ob/ob and lean mice in ligand binding within specific nuclei were observed as follows. β2-Adrenergic receptor autoradiography. The binding of [125I]CYP to β-adrenergic receptors was assessed by quantitative autoradiography in ob/ob (n = 5; body...
examined. The binding of \([^{125}\text{I}]\text{CYP}\) to \(\beta_1\)-adrenergic receptors, performed in the presence of the \(\beta_2\)-antagonist ICI-118551, was significantly increased among \(\text{ob/ob}\) mice in the PVN (by 30\%, \(P < 0.01\), LH (by 10\%, \(P < 0.05\)), and VMH (by 23\%, \(P < 0.01\)) compared with lean mice (Fig. 1A). Similarly, the binding of \([^{125}\text{I}]\text{CYP}\) to \(\beta_2\)-adrenergic receptors, performed in the presence of the \(\beta_2\)-antagonist CGP-20712A, was significantly increased among \(\text{ob/ob}\) mice in the PVN (by 30\%, \(P < 0.01\), LH (by 21\%, \(P < 0.05\)), and VMH (by 67\%, \(P < 0.001\)) compared with lean mice (Fig. 1B). No significant differences in \([^{125}\text{I}]\text{CYP}\) binding were seen in the DMH for \(\beta_1\)- or \(\beta_2\)-receptors or in the AH for \(\beta_1\)-receptors. In addition, \([^{125}\text{I}]\text{CYP}\) binding to \(\beta_2\)-receptors was on average \(~50\%\) of \([^{125}\text{I}]\text{CYP}\) binding to \(\beta_1\)-receptors.

\(\alpha_2\)-Adrenergic receptor autoradiography. The binding of \([^{125}\text{I}]\text{HEAT}\) to \(\alpha_2\)-adrenergic receptors was examined by quantitative autoradiography in \(\text{ob/ob}\) (\(n = 8\); body wt = 35.1 ± 3.3 g) and lean (\(n = 8\); body wt = 18.8 ± 0.9 g) mice. The same five areas within the hypothalamus were examined. The results were markedly similar to those seen for the \(\beta_1\) and \(\beta_2\)-adrenergic receptors. The binding of \([^{125}\text{I}]\text{HEAT}\) to \(\alpha_2\)-adrenergic receptors of \(\text{ob/ob}\) mice was significantly increased in the PVN (by 26\%, \(P < 0.01\)), LH (by 21\%, \(P < 0.05\)), and VMH (by 67\%, \(P < 0.001\)) compared with lean mice (Fig. 2). No significant differences in \(\alpha_2\)-binding were observed in the AH or DMH.

\(\alpha_2\)-Adrenergic receptor autoradiography. To complete our assessment of hypothalamic adrenergic receptors, the binding of \([^{125}\text{I}]\text{PIC}\) to \(\alpha_2\)-adrenergic receptors was assessed by quantitative autoradiography in \(\text{ob/ob}\) (\(n = 8\); body wt = 33.5 ± 3.1 g) and lean (\(n = 8\); body wt = 19.2 ± 1.0 g) mice. Again, the same five areas within the hypothalamus were examined. The binding of \([^{125}\text{I}]\text{PIC}\) to \(\alpha_2\)-adrenergic receptors in \(\text{ob/ob}\) mice, compared with lean mice, was significantly reduced in the VMH (by 37\%, \(P < 0.001\)) and DMH (by 12\%, \(P < 0.01\)) (Fig. 3). No significant differences were observed in the PVN, AH, or LH.

In an effort to determine whether this difference in \([^{125}\text{I}]\text{PIC}\) binding to \(\alpha_2\)-adrenergic receptors represented a lower density of \(\alpha_2\)-receptors in \(\text{ob/ob}\) mice or a difference in the affinity of the receptors for \([^{125}\text{I}]\text{PIC}\), saturation binding studies were performed. Saturable binding of \([^{125}\text{I}]\text{PIC}\) to \(\alpha_2\)-adrenergic receptors was achieved in the VMH and DMH of \(\text{ob/ob}\) (\(n = 8\); body wt = 18.4 ± 1.2 g) mice. Scatchard transformations of the data revealed that the affinity of \([^{125}\text{I}]\text{PIC}\) for \(\alpha_2\)-receptors was not significantly different in the VMH or DMH of \(\text{ob/ob}\) or lean mice (Fig. 4, A and B; Table 1). Values for \(B_{\text{max}}\), calculated from Scatchard transformations, however, indicated the density of \(\alpha_2\)-receptors in \(\text{ob/ob}\) mice to be significantly decreased in the VMH (by 39\%, \(P < 0.001\)) (Fig. 4, A and C; Table 1) and in the DMH (by 24\%, \(P < 0.05\)) (Fig. 4, B and C; Table 1).

To gain further insight into the observed differences in the density of \(\alpha_2\)-adrenergic receptors, subtype-spe-
Specific autoradiography was performed in the VMH and the DMH of ob/ob (n = 8; body wt = 35.1 ± 3.3 g) and lean (n = 8; body wt = 18.8 ± 0.9 g) mice. The decreased binding of [125I]PIC binding to $\alpha_2^*$ and $\alpha_{2A}^*$-adrenergic receptors in the VMH (Fig. 5A) and DMH (Fig. 5B) of ob/ob mice is apparent in the representative autoradiograms shown. In ob/ob mice, the binding of [125I]PIC to $\alpha_2^*$-receptors was significantly lower in the VMH (by 40%, $P < 0.001$) and DMH (by 23%, $P < 0.001$) compared with lean animals (Fig. 6, A and B), similar to the results reported in Fig. 3. Furthermore, in ob/ob mice, the binding of [125I]PIC to $\alpha_{2A}^*$-receptors, performed in the presence of the $\alpha_2$BC-antagonist prazosin, was significantly decreased in the VMH (by 47%, $P < 0.001$) (Fig. 6A) and in the DMH (by 23%,
the decrease in $\alpha_2$-subtype binding observed in $ob/ob$ vs. lean mice can be ascribed to the $\alpha_2A$-subtype. In lean mice, under these binding conditions, $\alpha_2A$-receptors represented 72% of the specific binding of $[^{125}\text{I}]$PIC to $\alpha_2$-adrenergic receptors in the VMH and 76% of the binding in the DMH.
DISCUSSION

This study is the first to delineate differences between obese-hyperglycemic, leptin-deficient (ob/ob), and lean-euglycemic (+/-) mice in noradrenergic ligand binding to discrete hypothalamic nuclei. Among ob/ob mice, the $\alpha_1$-, $\beta_1$-, and $\beta_2$-ligand binding are each increased in the VMH, LH, and PVN, whereas $\alpha_2$-ligand binding is decreased in the VMH and DMH relative to lean mice. Overall, the magnitude of these changes is most pronounced in the VMH. Taken together with several other studies of noradrenergic function within these nuclei and the consistent observation of increased hypothalamic NE levels in ob/ob mice (18, 41, 47), the present findings offer new insights into hypothalamic noradrenergic regulation of metabolism as follows.

In normal physiological states, increased NE postsynaptic receptor density (as observed in the VMH of ob/ob mice) is coupled to decreased presynaptic NE release (14). This hypersensitization comprises a compensatory response to decreased stimulus. However, available evidence suggests that in the metabolic syndrome (as in ob/ob mice), this “normal” neurophysiology is altered in the VMH and that the increased VMH postsynaptic NE receptor density thereof is not coupled to decreased NE release. Our results demonstrate a marked decrease in VMH $\alpha_{2A}$-receptor number in ob/ob vs. lean mice. In the hypothalamus, presynaptic NE $\alpha_2$-receptors are $\alpha_{2A}$, and NE activation of $\alpha_{2A}$-receptors is a primary inhibitor of presynaptic NE release (60). Thus the marked decrease in VMH $\alpha_{2A}$-receptors of ob/ob mice may potentiate an increase in NE release rate. Also, in ob/ob mice, the VMH NE levels are not decreased relative to lean animals but rather hypothalamic NE levels are increased (18, 41, 47). Although VMH NE release per se has not been quantified in ob/ob mice, largely due to logistical issues of microdialysis of such a small area, such studies have been conducted in other larger animal models of the metabolic syndrome. These studies indicate that an increased VMH extracellular NE turnover rate is associated with the metabolic syndrome (28, 42, 43).

Importantly, hyperinsulinemia, a hallmark of the metabolic syndrome (particularly so in ob/ob mice; 54) is per se a potent stimulus for VMH NE release (12). Hyperinsulinemia may induce this effect via reducing $\alpha_2$-receptor density (36), which is also a VMH characteristic of ob/ob mice. Therefore, available evidence indicates that the increased VMH NE postreceptor density characteristic of the metabolic syndrome as in ob/ob mice is not coupled to a decreased presynaptic NE release but rather possibly to an increased NE release.

The increased VMH noradrenergic ligand binding to $\alpha_1$, $\beta_1$, and $\beta_2$-receptors of ob/ob mice likely contributes to the increased electrophysiological responsiveness of the VMH to NE observed in these animals compared with lean controls (30). And, as mentioned above, the specific decrease in noradrenergic ligand binding to $\alpha_{2A}$-receptors in the VMH of ob/ob mice seen here can function to support increased levels of VMH NE release (60), as observed in other obese, glucose-intolerant animals (28, 43, 44). Therefore, the total changes in VMH NE receptor profile of ob/ob vs. lean mice facilitate increases in both postsynaptic levels of NE as well as responsiveness to NE. Such receptor-supported changes in VMH NE neurophysiology of ob/ob mice are in accordance with the observed induction of the obese, glucose-intolerant state after chronic infusion of NE in the VMH of normal animals (13, 40, 42, 59). In this regard, the relation of the present findings to VMH modulation of peripheral lipid and glucose metabolism must be addressed.

Chronic infusion of NE into the VMH stimulates fattening by increasing white adipose lipogenesis and decreasing brown fat energy expenditure without inducing hyperphagia (13, 59). Glutamate activation of VMH neurons stimulates SNS activation of brown fat (2, 66). The effect of iontophoretically applied NE to inhibit glutamate-evoked neuronal activity within the VMH is markedly enhanced in ob/ob vs. lean mice (30). Consequently, in ob/ob mice the SNS drive for brown fat thermogenesis is reduced (67), thereby supporting obesity. The present findings suggest that $\alpha_1$- and $\beta$-receptors may be involved in this augmented response to NE in ob/ob mice. In addition, acute and chronic infusion of NE into the VMH each increase plasma insulin level (13, 16, 59, 62), which is the most potent lipogenic stimulus known. Therefore, the VMH...
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noradrenergic receptor profile of ob/ob mice may contribute to the profound hyperinsulinemia and subsequent obesity of these animals. Considering glucose metabolism, it is well established that acute administration of NE into the VMH of normal animals quickly raises plasma glucose as well as glucagon, NE, and insulin levels (16, 22, 56–58, 62). The VMH is a glucose sensor able to induce, via NE release and subsequent stimulation of the SNS, rapid and marked increases in plasma glucose, via increased hepatic glucose output (HGO), in response to systemic or local glucopenia (4, 8, 9, 16, 51). Furthermore, hypoglycemia stimulates decreases in medial α2-receptors (11) that in turn increase postsynaptic NE levels (4, 60) to facilitate the glucose counterregulatory response (22). In normal animals, however, local VMH hyperglycemia blocks this VMH response to systemic hypoglycemia (8) and there is a positive correlation between plasma glucose level and VMH (and DMH) α2-ligand binding (11, 37). That is, high glucose levels turn off the VMH NE drive for increased HGO. As such, the hyperglycemia of ob/ob mice is coupled with an inability of the VMH to appropriately sense and respond to it (i.e., high glucose coupled to decreased VMH α2-receptor number). In fact, the VMH NE receptor profile of ob/ob mice supports increased NE activity, which in turn potentiates hyperglycemia. It is as if the VMH of hyperglycemic ob/ob mice is sensing and responding to hypoglycemia. Likewise, diet-induced obesity (DIO)-prone rats also have a loss of normal VMH α2-receptor and neuronal responsiveness to glucose (35, 37). Moreover, decreased α2A-binding in the DMH of ob/ob mice can facilitate SNS activity and increased HGO (3). It should be understood that the reported decreased SNS activity of ob/ob mice refers to brown fat activity and not control of HGO (67). And, as discussed herein, increased hypothalamic NE activities decrease SNS input to brown fat to reduce energy expenditure and increase SNS drive to liver (via neural and endocrine routes) to increase HGO.

There is one final and important note regarding the decreased α2-receptor binding in the VMH and DMH of ob/ob mice. That is, the hyperinsulinemia of these mice (and of obese, glucose-intolerant animals in general) may reduce medial α2-ligand binding (12, 36) and thereby maintain the medial hypothalamic stimulation of this condition.

The VMH is a primary site of leptin action to reduce obesity and improve glycemic control (17, 26, 45), which are opposite to the actions of NE therein (13, 40, 42, 59). The absence of leptin in ob/ob mice may permit the observed VMH NE receptor changes that potentiate the obese-hyperglycemic state. As a consequence of their leptin deficiency, ob/ob mice cannot appropriately assess and/or modulate energy balance and peripheral metabolism. Essentially, these animals are in a chronic fattening mode, never sensing their obesity (19, 25). In this regard, it appears relevant that the DIO-prone rat also exhibits decreased VMH α2-binding [assessed by paraminoclonidine binding, which exhibits some selectivity for α2A-receptors (1)] before developing obesity (33, 34). Moreover, these animals can be prospectively identified by their increased systemic noradrenergic (SNS) response to intravenous glucose administration (39). Therefore, in both the ob/ob mouse and the DIO-prone rat, the state of fattening (or susceptibility to fattening) is coupled to (and likely in part a result of) decreased VMH α2-binding, thereby potentiating NE activities therein, which in turn can induce the obese, glucose-intolerant condition (13, 40, 42, 59). Importantly, once obesity is achieved in the DIO rat, the VMH α2-receptors become increased (32) and α1-receptors become decreased (65), relative to lean controls, due to an autoregulatory response (allowing for a new steady state of metabolism, i.e., termination of fattening), which is diminished in leptin-deficient ob/ob mice. Collectively, the above discussion indicates that the observed decreased noradrenergic binding to α2A-receptors (likely presynaptic) in the VMH (and DMH) and the increased noradrenergic binding to α1A, β1, and β2-receptors therein contribute to the obese, glucose-intolerant state of ob/ob mice and support the postulate that increased VMH NE activity potentiates the development of this metabolic syndrome.

Regarding the PVN, noradrenergic binding to β (and to some extent α1)-receptors is known to be a strong stimulus for corticotropin-releasing factor (CRF) secretion (50, 53). Thus the increase in noradrenergic binding to α1A, β1, and β2-receptors in the PVN of ob/ob mice may contribute to the increased PVN CRF content and hypothalamic-pituitary-adrenal axis overactivation characteristic of these obese glucose-intolerant mice (5, 6, 24). Such noradrenergic receptor changes within the PVN of ob/ob mice may also contribute to the increases in CRF within PVN terminals in the DMH (5) that function to activate the SNS innervation of liver and white adipose and thereby increase plasma glucose and FFA levels (3, 6) typical in these mice (54, 55).

Such increased noradrenergic binding may well also influence other neuropeptide secretions and neuronal communications within the PVN. PVN postsynaptic α2-receptors are known to mediate the noradrenergic drive for feeding during the daily nocturnal feeding cycle in rodents (31), so it may seem surprising that no change in PVN [125I]PIC binding was observed in hyperphagic ob/ob vs. lean mice in this study. It must be appreciated, however, that in this study neither PVN postsynaptic α2-binding was specifically assayed nor was PVN tissue obtained during the feeding cycle (but rather during the fasting period of the day: 4 HALO).

With respect to the LH, a primary adrenergic response at this site is an increase in circulating insulin (61, 63). Given that the obese, glucose-intolerant condition is characterized by hyperinsulinemia (16), especially in ob/ob mice (54), the increased binding to α1A, β1, and β2-receptors observed in the LH of ob/ob mice may contribute to the hyperinsulinemia. Similar increases in α1A-binding in the PVN and LH as those observed here for ob/ob mice have been reported in Zucker fa/fa rats, although some heterogeneity of noradrenergic receptor profile among other hypothalamic
sites exists between the two animal models (27, 38) possibly due to differences in animal age, sex, and body adiposity at the time of analysis.

Perspectives

Hypothalamic (VMH) NE levels and/or release have been demonstrated to be elevated in a wide variety of animal models of the metabolic syndrome, including ob/ob mice (18, 20, 28, 41, 43, 44, 47). The present study has identified increased noradrenergic ligand binding densities for \( \alpha_1 \), \( \beta_1 \), and \( \beta_2 \)-receptors in the PVN, VMH, and LH and decreased \( \alpha_2 \)-binding in the VMH and DMH. These receptor changes support increased postsynaptic binding of NE as well as the presynaptic release of NE, particularly in the VMH. That is, the normal neurophysiological regulation of synaptic NE neurotransmitter action (i.e., reciprocal modulation of neurotransmitter release and postsynaptic receptor density) is absent in the metabolic syndrome of these ob/ob mice and likely in other animal models of the syndrome as well (33, 35, 37, 44). Furthermore, other studies wherein NE has been chronically infused into the VMH of normal animals have clearly demonstrated that increased VMH NE synaptic level is not merely associated with, but actually causative in, the development of the full-blown metabolic syndrome (obese, hyperinsulinemic, glucose-intolerant state) (13, 40, 42, 59). The abovementioned observed changes in hypothalamic NE binding of ob/ob mice support I) increased SNS stimulation of HGO, FFA release, and glucagon secretion (16, 22, 56–58, 62); 2) inhibition of VMH-SNS activation of brown fat thermogenesis (2, 30, 66); and 3) parasympathetic stimulation of insulin secretion (61, 63); all correlates of the metabolic syndrome.

How then is this “abnormal” hypothalamic noradrenergic neuronal circuitry of the metabolic syndrome generated? An obvious culprit in the ob/ob mouse is the genetic lack of leptin (and possibly leptin resistance in other model systems). Interestingly, the leptin effects in the VMH are counter to those of NE (17, 26, 45). And, VMH infusion of NE generates hyperleptinemia, leptin resistance, and the metabolic syndrome (13). However, in seasonal animals that develop and reverse the metabolic syndrome as part of an annual cycle of metabolism, the metabolic syndrome is also associated with increased VMH NE activity, indicating that this “altered” hypothalamic NE neurophysiology is natural (not abnormal), malleable, and, most importantly, reversible (44). Efforts to understand the modulation of this hypothalamic NE response system regulating metabolism should lead to a new understanding of the causes and possible therapeutic targets of the metabolic syndrome.

In conclusion, hypothalamic noradrenergic receptor differences in conjunction with increased hypothalamic NE levels in ob/ob vs. lean mice support the obese, hyperglycemic, and hyperinsulinemic state observed in these animals. Moreover, these findings coupled with numerous previous studies of hypothalamic noradrenergic function indicate that such a unique neurophysiological consequence of simultaneously increased noradrenergic stimulus and response systems within the hypothalamus may be a general feature potentiating the pathophysiology of the obese, glucose-intolerant condition.

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