

The Brain and the Metabolic Syndrome: Not a Wireless Connection

The metabolic syndrome is currently a major health threat (1). It is defined by a combination of obesity, insulin resistance, hypertension, and lipid disorders (2), and many cases are believed to be a consequence of diet-induced obesity. Peripheral organs involved in the early phases of the development of the metabolic syndrome include the pancreas, liver, and adipose tissue. Progress in the understanding of obesity and diabetes obtained over the last decade has revealed multiple pathways interconnecting these peripheral organs with the central nervous system (CNS), emphasizing an important role for the brain in the physiology as well as pathophysiology of peripheral energy balance and glucose homeostasis (3). According to a currently widely accepted model (4–6), specific circuitry in the CNS continuously receives afferent information regarding the status of peripheral metabolism via hormonal signaling as well as via direct macronutrient sensing. The center of these CNS networks is believed to be situated within the mediobasal hypothalamus and to be highly interconnected with brainstem areas and the mesolimbic reward circuitry. Afferent signals targeting these central areas include adipocyte factors (such as leptin), pancreatic hormones (such as insulin), and macronutrients (such as glucose and long chain fatty acids), which are, at least in part, produced by the liver.

Although this model (4–6) clearly reflects the intriguing overlap among essential components of energy balance regulatory pathways with the main organs known to be involved in the pathophysiology of the metabolic syndrome, it is necessarily oversimplified. As an example, one additional mechanism informing the CNS about peripheral nutrient and energy balance status is represented by legions of afferent gastrointestinal signals (7). Similarly, besides being produced by the liver, macronutrients are also directly absorbed from the intestine. Moreover, secretion of the adipokine leptin is not limited to the visceral adipose depots that are believed to be involved in the pathophysiology of the metabolic syndrome. Subcutaneous adipose depots, which are believed to play a much less important role for the development of obesity and diabetes (8), also produce and secrete leptin (9), as does the stomach. If the metabolic syndrome were to be caused by a single impairment within the endocrine signaling and/or nutrient sensing systems known to regulate energy homeostasis, functional or anatomical connections among liver, pancreas, and visceral fat should reflect the changes and initiate compensation.

Abbreviations: ANS, Autonomic nervous system; CCK, cholecystokinin; CNS, central nervous system; DMV, dorsal motor nucleus of the vagus; PVN, paraventricular nucleus; SCN, suprachiasmatic nucleus; WAT, white adipose tissue.

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In a notable study published in this issue of *Endocrinology*, Kreier *et al.* (10) remind us that, apart from endocrine signaling and nutrient sensing, there is an important neuronal network that connects the CNS with peripheral metabolic processes. They demonstrate for the first time that the liver, the pancreas, and the visceral adipose tissue—but not the sc adipose tissue—share a common and specific neuronal connection with the CNS. Intriguingly, this hardwired link can be traced through several brain regions without indication of it interacting with other neuronal projections to or from peripheral organs.

The use of viruses for the labeling and tracing of interconnected neurons at various CNS hierarchic levels allows for a highly detailed anatomical analysis of functionally discrete circuits within the autonomic nervous system (ANS) (11). Importantly, the specific CNS areas known to control the ANS have become a particular focus of recent research studying the regulation of fuel storage and use. Indeed, recent anatomical observations indicate that CNS neuronal circuits responsible for sensing peripheral signals relevant to energy balance regulation are highly interconnected with neural projections to and from metabolically important peripheral organs (12, 13). In addition, there is evidence that, as the levels of various peripheral signals change, specific neuronal circuits within the CNS respond by adjusting ongoing ANS activity to a wide spectrum of organs. For example, leptin, which primarily targets several of CNS areas, can increase the expression of uncoupling protein 1 in brown adipose tissue via activation of the sympathetic nervous system (14). Leptin has also been reported to increase sympathetic nerve activity by activating hypothalamic melanocortin-4 receptors, most likely via stimulation of proopiomelanocortin expressing neurons of the arcuate nucleus (15).

One of the current scientific tasks is to identify which exact CNS nuclei play an essential role in integrating the wide array of energy-balance afferent signals, to generate coordinated patterns of homeostatic ANS outputs, as well as how these regulatory areas interact. One particular neuronal population that appears to link the energy balance regulatory system with the ANS is in the region of the paraventricular nucleus (PVN) (13, 16), which contributes to the regulation of several homeostatic functions including fluid balance and cardiovascular homeostasis (17, 18). Based on its rich, bidirectional connectivity with specific sympathetic and parasympathetic nuclei in the brainstem and spinal cord, as well as its direct control of endocrine and neuroendocrine outputs (19), the PVN stands as a key candidate area within the CNS to play a major role in the coordination of complex patterns of homeostatic responses to peripheral signals related to energy balance and metabolism. Expression of melanocortin-4 receptors, which are known to play a core role in energy balance regulation (20), have been localized on PVN neurons

which have been identified as part of the ANS by injecting pseudorabies virus as a tracer in the white adipose tissue (WAT) (13). Consistent with this, the activity of PVN neurons projecting to the sympathetic nervous system can be modulated by leptin acting in the arcuate nucleus, by stimulating the release of α -MSH in the PVN (21).

Another CNS area of interest for such a link is the suprachiasmatic nucleus (SCN), which sends information regarding circadian rhythms to numerous hypothalamic centers and other brain regions (22). A neuroanatomical link between peripheral tissues such as WAT (23) and liver (24) with the SCN has been demonstrated with elegant viral tracing techniques, indicating that the ANS directly transfers circadian information to its cellular targets at peripheral organs. Interestingly, mice with a mutated *clock* gene, which encodes for a transcription factor expressed in pacemaker neurons in the SCN, have characteristic alterations reminiscent of the metabolic syndrome, including hyperglycemia, hyperlipidemia, and obesity (25).

The role of brainstem structures integrating energy balance signals with autonomic responses has been thoroughly investigated. Functional roles of this area include a major responsibility for the sympatho-adrenal response to glucoprivation (26). Neuronal circuitry in the brainstem is intimately involved in the regulation of energy balance and is, for example, a direct target area for both the satiety-inducing hormone leptin (27) and the hunger-inducing hormone ghrelin (28).

In this issue of *Endocrinology*, Kreier *et al.* (10) report new findings emphasizing the relevance of interactions between the CNS and organs directly involved in the metabolic syndrome. They demonstrate that injections of neuronal tracers in visceral fat label neurons in the spinal cord, brainstem, and the hypothalamus, and in some cases also within the gracilis nucleus. As cogently pointed out by Bartness *et al.* (29), such data confirm previous findings by Fishman and Dark (30) who had reported that the anterograde tracer *True Blue*, administered into WAT, labels neurons projecting to dorsal root ganglia. Therefore, work originating from different laboratories supports an anatomical connection between visceral adipose tissue and nociceptive sensing structures. In addition, substance P, a molecular marker of sensory neurons, has also been localized in WAT (29). Kreier *et al.* (10) hypothesize that the sensory afferents projecting to the gracilis nucleus could be involved in nociceptive processes such as sensing of mechanical stress in adipocytes. It seems to us, however, that if increasing adipocyte size were actually painful, obesity might not have become the epidemiological threat it currently is. Further studies are needed to understand the role of such a sensorial innervation in adipose tissue.

Nonetheless, the present findings support the idea that, in addition to humoral factors, the metabolic status of the adipose tissue can be conveyed to the CNS via hardwired connections. Exactly how and where the CNS processes information acquired from these sensory nerve endings, and how both humoral and neuronal afferent inputs are integrated with them to elaborate complex homeostatic responses, are important questions that remain to be addressed. Hypothetically, interactions among peripheral and

neuronal afferent signals conveying information about the status of energy balance could occur both at central and at peripheral loci (see Fig. 1). For example, the activity of afferent peripheral nerve terminals is amenable to modulation by circulating hormones. In this sense, it was previously reported that the injection of leptin into epididymal fat increases the afferent nerve activity from that fat pad (31). The fact that these sensing nerve terminals in the adipose tissue could receive hormonal information and adjust activity accordingly is reminiscent of sensitive fibers innervating the gastrointestinal tract that can be stimulated by the gastric hormone cholecystikinin as well as by gastric distension (32). Similarly, neurons from the nodose ganglion (where the soma of the vagal afferent neurons are localized) express ghrelin, leptin and cholecystikinin, receptors, among many others (33).

Using two different fluorescent markers, one injected in liver and the other in pancreas, Kreier *et al.* (10) observe colocalization of both markers in the same neurons in the dorsal motor nucleus of the vagus (DMV), demonstrating that both organs seem to share an identical vagal innervation. Interestingly, when tracers were injected into sympathectomized liver (thereby leaving the vagus nerve as the only hardwired hepatic connection to the CNS) and retroperitoneal fat, colocalization of both signals was observed in vagal motor neurons. This demonstrates that liver and visceral fat also share overlapping parasympathetic innervation as well. It has also been reported that insulin-sensitive neurons in the hypothalamus control hepatic glucose production (34) via the vagus nerve as well (12).

Using pseudorabies virus as a transneuronal tracer, Kreier *et al.* (10) identified specific sympathetic and presympathetic

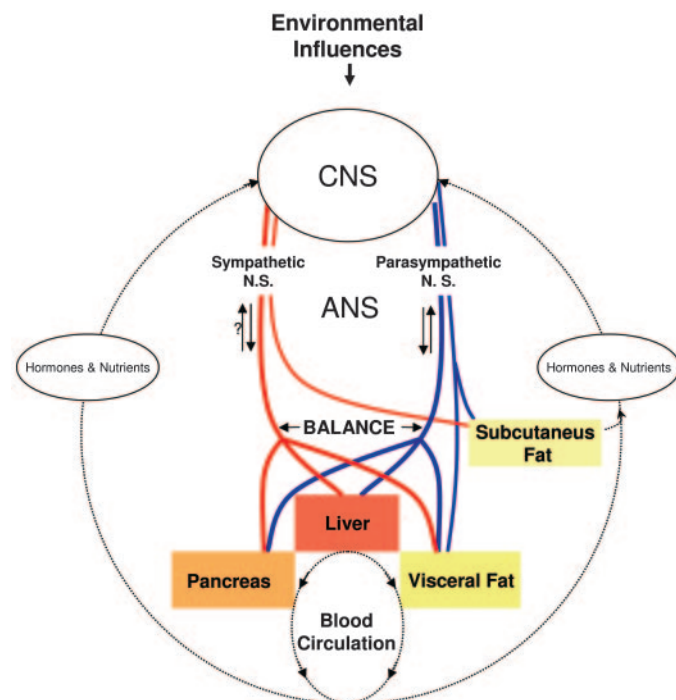


FIG. 1. Interactions among peripheral and neuronal afferent signals conveying information about the status of energy balance could occur both at central and at peripheral loci.

neuronal pathways innervating different fat compartments. However, there are some important caveats that need to be taken into consideration when attempting to infer direct connectivity and hierarchical organization among various CNS nuclei based on this methodological approach. For example, labeling neuronal pathways of progressively higher hierarchical order is largely dependent on the time course after the initial injection of the tracer, being increasingly less specific at longer intervals. In this sense, the lack of detailed information regarding the precise sampling timing within the different brain regions studied, along with a lack of a systematic quantitative analysis of single/double-labeled neurons within these regions, limits the overall interpretation of the results reported.

Despite these concerns, the results presented by Kreier *et al.* (10) are generally supportive of a somatotopic organization of a sympathetic innervation according to specific fat depots (*e.g.* retroperitoneal *vs.* sc), an organizational pattern that appears to be preserved even at high hierarchic-order brain regions, such as the PVN, the medial preoptic area, the SCN and the amygdala. In view of the findings of Kreier *et al.* (10), a segregated, fat compartment-specific organization appears to exist for the parasympathetic nervous system as well—at least at the level of the DMV. It will be important to determine in future studies whether a similar somatotopic organization to that described for the sympathetic limb also occurs at higher preautonomic centers projecting to the DMV.

Importantly, both anatomical and functional studies indicate that in addition to innervating brainstem and spinal cord sympathetic centers, subsets of preautonomic neurons in the PVN also project directly to, and regulate the activity of, neurons in the DMV (35). Thus, it is likely that presympathetic and preparasympathetic neurons within homeostatic CNS centers, such as the PVN, are topographically and functionally organized within discrete neuronal circuits according to specific organs. Coordinated activity within such discrete PVN circuits may then play a key role in generating a balanced autonomic output to the liver, pancreas and adipose tissue.

Based on the current and previous studies, Kreier *et al.* (10, 36, 37) propose the intriguing hypothesis that a loss of the normal physiological balance between sympathetic and parasympathetic outputs is a major factor that could promote the development of the metabolic syndrome. However, whether an impairment of the activity of ANS in liver, pancreas, or adipose tissue has a major impact in the development of metabolic syndrome, or actually leads to hyperinsulinemia or diabetes, remains to be demonstrated. Parasympathetic denervation of retroperitoneal fat pads, for example, increases activity of the enzyme, hormone-sensitive lipase and decreases uptake of glucose and free fatty acids in adipocytes. These findings support a functional consequence of an altered autonomic balance in the sense that the sympathetic tone dominates due to decreased parasympathetic tone (36). However, there is still no convincing evidence that the ANS actually plays a causal role in the development of any part of the metabolic syndrome. In other words, and as is many times the case, despite an intriguing association and multiple links between two phe-

nomena, it is unclear which one is the “chicken” and which one is the “egg.”

The anatomical and functional links among brain areas controlling energy balance and areas controlling ANS activity introduce overlapping pathways that make a detailed analysis of the role of each independent player controlling energy balance difficult. Recently, Morton *et al.* (38) have proposed that the sensing of leptin in the hypothalamus modulates the effect of the vagal afferent satiety signal triggered by CCK. Thus, the anorectic effect of cholecystokinin (CCK), absent in leptin receptor-deficient rats, can be recovered after the viral induction of the leptin receptor expression specifically in the arcuate nucleus. Apart from that, recent *in vitro* studies in adipocytes add another level of complexity. Zhang *et al.* (39) have demonstrated that insulin impairs the isoproterenol-induced activation of protein kinase A and phosphorylation of endogenous cAMP response element binding protein, suggesting that insulin pathways can establish a heterologous cross talk with intracellular β -adrenergic signaling. Thus, it seems unlikely that the development of the metabolic syndrome would exclusively depend on alterations of ANS balance, as proposed by Kreier *et al.* (10), without complex interactions and feedback mechanisms of numerous other endocrine and metabolic systems of which the insulin pathway is a single player. Moreover, a potential role for the described neuronal pathways in the pathophysiology of type 2 diabetes may be slightly overemphasized in this study, at least in view of the currently available data. Further studies employing animal models of diabetes and obesity should allow for testing the intriguing hypothesis proposed by Kreier *et al.* (10). Pancreas, liver, and fat tissue undoubtedly all play a role in type 2 diabetes and obesity (as they do for the pathophysiology of many other diseases). However, without actual data from animal models of type 2 diabetes or obesity supporting a functional relevance of the proposed signaling pathways presented here, the overall role of these pathways in type 2 diabetes and related disease states should be interpreted cautiously.

In summary, the present work by Kreier *et al.* (10) further supports an emerging model in which a rich bidirectional neural communication between the CNS and peripheral organs plays a key role in the control of peripheral metabolism. In this scenario, the status of energy balance is conveyed to the CNS by means of circulating humoral factors, nutrients, and neuronal afferent inputs. In turn, the CNS can modulate peripheral metabolism by regulating the activity of hypothalamic-hypophyseal axes, and as supported by this study, in parallel modify tissue-specific metabolism by modulating the balance between sympathetic and parasympathetic outputs. Based on recent findings as cited above (38, 39), interactions among humoral and neural signals at the various hierarchical levels of this system may allow for precise fine-tuning of the input-output function of the energy balance regulatory circuitry (see Fig. 1). Finally, external stimuli, such as circadian timing (40), olfactory or taste inputs, and complex processed information derived from hippocampal or cortical areas (4), certainly add another level of modulatory influence. These inputs allow the brain to synchronize the activity of the homeostatic regulatory system with environ-

mental challenges or cognitive influences on the energy balance control center and can modulate the ANS as well.

Whether the newly identified signaling pathways play a role in the development of the metabolic syndrome remains to be investigated. One insight, however, already has emerged: the “wireless age” does not extend to the cross talks of metabolic physiology and its pathological derangements.

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