Heart hormones fueling a fire in fat

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Our view of how adipose tissue metabolism is regulated recently experienced a change in focus and breadth, meaning that some of the key controlling factors were not fully in the picture. The catecholamines of the sympathetic nervous system are well-known activators of β-adrenergic receptors in adipocytes to increase lipolysis. They also drive energy expenditure in brown adipose tissue and, importantly, the “browning” of cells in white adipose depots. However, this is clearly not the whole story. In earlier work, we established a pathway from β-adrenergic receptors to p38 MAP kinase to drive the transcription of brown adipocyte genes and respiratory uncoupling. Now we recently discovered that cardiac natriuretic peptides (NPs) stimulate a similar “browning” of human and mouse adipocytes. NPs activate the guanylyl cyclase coupled NP receptor A and activation of protein kinase G. Importantly, this pathway also depends upon p38 MAPK. These two pathways work together, additively increasing expression of brown adipocyte marker genes, as well as reflexively controlling each other’s components. We discuss these findings and how the control of body fat by these cardiac hormones, in conjunction with the sympathetic nervous system, has implications for obesity as well as cardiovascular disease, including hypertension and heart failure.

The pathway from the sympathetic nervous system to β-adrenergic receptors (βARs) and protein kinase A (PKA) was well established in earlier decades to be the key controlling element for activating the enzymatic apparatus to hydrolyze stored triglyceride in adipocytes1-3 (see upper part of Fig. 1). With the realization that brown adipose tissue was a thermogenic form of fat4-6 due to the unique “uncoupling protein” (UCP1) of the brown adipocyte (see ref. 8 for further details), it was subsequently shown that the βAR-PKA pathway was also responsible for the gene expression changes and expansion of brown adipocytes for respiratory uncoupling and energy expenditure. Interest in studying brown adipose tissue as a sink for excess calories has experienced several waves of enthusiasm and disdain over the decades, falling in and out of fashion depending upon whether it was considered clinically relevant or not. At the moment, brown adipocytes in humans are in vogue again as a result of radiology studies showing cold-stimulated bilateral glucose uptake in adult humans and biopsies from subjects demonstrating UCP1-positive brown adipocytes.9-13

Our lab has studied the βARs and their signaling mechanisms in adipocytes for several years, focusing on cAMP, PKA and downstream targets of the kinase such as enzymes and transcription factors14,15. We became particularly interested in the transcriptional activation of the Ucp1 gene in response to β-agonist stimulation. Although the Ucp1 genes from various species had been isolated in the early 1990s and regulatory regions responsive to elevations in cAMP were identified16-18, it seemed curious that relatively little progress had been made in identifying the transcription factors that controlled cAMP-dependent Ucp1 expression. It was only when we stumbled upon the PKA dependent activation of p38 MAPK19,20

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that pieces of the puzzle began to be put into place. Discovering that p38 MAPK activation by βARs and PKA was necessary for driving Ucp1 gene transcription was a breakthrough in beginning to crack the code for how the SNS could stoke UCP1-dependent adaptive thermogenesis.

As shown in Figure 1, p38α MAPK (p38) is activated by a cascade downstream of PKA. The exact players in this cascade are not fully understood and their identity is a topic of ongoing work. However, functionally it is clear that p38 plays an important role at several levels: by phosphorylating PGC-1α, which boosts it co-activator function, as well as phosphorylating the CRE-binding factor ATF2 in order to drive expression of PGC-1α itself. There are several other known and suggested transcription factors involved in orchestrating Ucp1 gene transcription at this rich enhancer region of the gene, but whether p38 or other kinases are involved in their control has not been fully investigated. Indeed the full picture of all the factors and the signals that promote their assembly at this gene has yet to be constructed.

Our recently published study demonstrating that cardiac NPs ANP (atrial NP) and BNP (B-type-NP) increase the expression of brown adipocyte genes and uncoupled respiration, which is the topic of this commentary, arose from intersecting interests: one of us (S.C.) was focused on the SNS and molecular signaling and the other (M.B.) comes from a more clinical research background on obesity and hypertension.

The discovery of the natriuretic peptides produced and secreted from the heart was a major change in the mind-set about the function of the heart: it was not only a contractile “pump” for blood circulation but a bona fide endocrine organ. The NP system is integral to the control of blood pressure through its stimulation of fluid and salt release by the kidney. Two receptors tightly control the response to the NPs: the signaling form of the receptor termed NPRA/NPR1/GC-A and the clearance form of the receptor termed NPRC/NPR3/ANPRC (Fig. 2). Almost two decades ago, NP receptors were unexpectedly found to be expressed in adipose tissue of both rats and humans and, interestingly, levels of NPRC in rat adipose tissue were found to be sharply decreased by fasting. Together, these were some of the first results to suggest that perhaps cardiac NPs have a metabolic role in adipocytes, including a putative role for adipose tissue in the clearance of these peptides from the circulation. It was then reported that ANP could increase lipolysis in human adipocytes, but that this property did not exist in rodent adipocytes. Why did this process not exist in rodent adipocytes? Experimental observations as well as inspecting the Novartis Institute gene expression database (www.biogps.org) showed that mouse adipose tissue expresses vastly more NPRC than human adipocytes. What if these levels could be modulated? Would that affect the response? Mice with targeted disruptions of the Npra and Nprc genes had been generated more than a decade earlier. However they had not been studied from the point of view of body composition and adipose tissue. We did notice in the literature, however, that mice with spontaneous loss-of-function mutations in the Nprr gene were referred to by names like “longjohn” and “strigosus,” the latter from the Latin meaning “long and emaciated.” We decided to obtain these mice, which proved valuable to our studies.

We discovered that mice lacking NPRC were exceedingly lean and chock-full of brown adipocytes. By culturing their white and brown adipocytes it was easily observed that these murine cells could readily respond to ANP to increase lipolysis as well as turn on the brown adipocyte gene program. Our collaborator Nobu Takahashi provided his findings that Npra-/- mice tended to be obese. Thus we knew that it was not simply a primate-specific signaling mechanism, rather it is the ratio of NPRA/NPRC that dictates the response to NPs in adipocytes. As we went on to show and discuss below, the levels of these receptors and their response to NPs are quite dynamically regulated in mice as a normal course of their physiology. There may also be differences in responsiveness among different inbred strains of mice.

In order to better provide some physiological evidence of the role of NPs in driving both the “browning” of adipocytes within the white adipose tissue (WAT) depots and thermogenesis in brown adipose tissue (BAT), we set up a cold challenge experiment using wild-type mice. Part of the idea was that if fasting in mice was associated with a decrease in adipose tissue levels of NPRC, and blood pressure is increased in response to cold exposure, then maybe we would see an effect on the NP system in this experiment. This
was indeed the case.\textsuperscript{23} In fact, blood levels of BNP were markedly higher in the mice kept at 5°C for 6 h. Perhaps this indicates that the SNS is promoting the secretion of these hormones from the heart as well as increasing the expression of their genes. How this cold-exposure maneuver has this effect mechanistically is not clear, but it may be related to the cold induced increase in blood pressure seen in rodents and humans.\textsuperscript{34,36-38} In these experiments we were also gratified to see that in both the interscapular BAT as well as in WAT depots, such as gonadal and inguinal, the expression levels of NPRA increased in response to the cold challenge, while NPRC levels were decreased. Together these results suggest, although certainly do not prove, that the SNS is modulating the NP system in order to set up conditions that favor enhanced signaling by the NPs to coordinate with the SNS to drive both fuel mobilization (lipolysis) as well as ramping up the machinery for adaptive thermogenesis. In conjunction with these mouse studies we used human multipotent adipocytes that can differentiate into a cell with both “white” and “brown” characteristics, and we quickly saw that the whole program of brown adipocyte gene expression and function was getting activated, and that the longer the stimulus the greater the response.\textsuperscript{23} The notion that the SNS and NP systems work together was further strengthened by the finding that low concentrations of ANP and the β-agonist isoproterenol work together in culture human adipocytes. So together with the in vivo data there seems to be a coordinated and dynamic regulation between the SNS and the NP systems. Whether the reciprocal regulation (i.e., NPs regulating SNS components in adipose tissue) exists needs to be investigated. Although we indeed observed that delivery of BNP itself via minipump (7 d) to mice resulted in increased energy expenditure and increased expression of all the brown adipocyte markers—especially in WAT,\textsuperscript{23} we did not examine whether this elevation also had effects on receptors and responses of the SNS. It will be important to understand this regulation in more detail as we tease apart the molecular mechanisms involved in this apparent cross-talk between these hormonal systems.

Since these cell culture and animal studies demonstrate the parallel pathways of the SNS and the NP system to regulate fat metabolism (Fig. 3), it may also shed some light on earlier perplexing studies in genetically modified mice. For example, Palmiter and colleagues reported that mice with targeted deletion of dopamine β-hydroxylase, which lack the ability to synthesize epinephrine and norepinephrine,\textsuperscript{39} were not prone to excess weight gain on a high-fat diet. The fact that they were also hypotensive\textsuperscript{40} might suggest that the NP system at some level is compensating in their adipocytes for the lack of SNS drive. Thinking more broadly about the implications of our findings, there is obviously a strong connection between obesity and hypertension. Earlier work on the NP system identified peptides that bound NPRC but not NPRA\textsuperscript{41} and conversely other reagents that favor interaction with NPRA were also identified.\textsuperscript{32} If therapeutic approaches could be developed that target NPRA—or attempt to block NPRC—these could serve to both promote fat oxidation and weight loss by increasing amounts of brown adipocytes, as well as reducing blood pressure. Obviously there is much additional work to be done in the basic research lab as well as in the clinical setting to test whether such an approach is viable. From another perspective, as we noted in our study, conditions such as heart failure could be a situation where one might want to interfere with adipose tissue NP signaling. This notion stems from the well-established observations of weight loss that occurs in heart failure, known as pathological cardiac cachexia. High levels of NPs are characteristic of heart failure and are used as diagnostic markers of disease severity.\textsuperscript{43,44} Cardiac cachexia is a commonly encountered clinical problem\textsuperscript{45,46} and is associated with a poor prognosis.\textsuperscript{47} Less well studied but deserving further investigation is the pathological weight loss associated with anorexia nervosa (AN), which in some reports is correlated with elevated levels of circulating NPs,\textsuperscript{48} and they are reported to also have increased diet-induced thermogenesis\textsuperscript{49} and large caloric requirements to gain weight.\textsuperscript{50,51} If such a correlation were strengthened, this might be a predisposing factor for AN, or a co-morbid state due to the excessive exercise that anorectics engage in. In either of these cases an attractive hypothesis that we are now pursuing—particularly for heart failure—is that the pathological weight loss associated with high circulating NPs and/or catecholamine levels may involve increased brown fat and energy expenditure.

For those of us who have focused for many years on the role of the SNS in regulating adipose tissue lipolysis and the expansion and activity of brown fat for adaptive thermogenesis, the realization that the NP system has been functioning in a parallel manner without our considering it is like discovering an elephant in the room. Now with this elephant clearly in our line of sight, our studies of adipose tissue metabolism will never be the same.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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