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β-Adrenergic Receptors and Adipose Tissue Metabolism: Evolution of an Old Story

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Abstract

The role of β-adrenergic receptors (βARs) in adipose tissue to promote lipolysis and the release of fatty acids and nonshivering thermogenesis in brown fat has been studied for so many decades that one would think there is nothing left to discover. With the rediscovery of brown fat in humans and renewed interest in UCP1 and uncoupled mitochondrial respiration, it seems that a review of adipose tissue as an organ, pivotal observations, and the investigators who made them would be instructive to understanding where the field stands now. The discovery of the β_3-adrenergic receptor was important for accurately defining the pharmacology of the adipocyte, while the clinical targeting of this receptor for obesity and metabolic disease has had its highs and lows. Many questions still remain about how βARs regulate adipocyte metabolism and the signaling molecules through which they do it.

INTRODUCTION

Since the time of the earliest anatomists, such as Galen and Vesalius, the nature of stored fat in the body has been passionately debated (see 1, 2) as to whether it was actually a distinct tissue, a gland, or just lipids that accumulated in collagen-containing areas as a result of adequate or overnutrition. In addition, what we now call brown adipose tissue, about which currently there is much interest and enthusiasm, was hotly contested as to whether it was a part of the thymus or some type of endocrine gland involved in blood formation, among others (3). It also became referred to as the hibernating gland because of studies in hibernators (4). Its thermogenic properties were finally described by Smith (5) and Ball & Jungas (6). I became interested in adipose tissue and adipocytes because I wondered why some cells express more than one β-adrenergic receptor (βAR), when it seemed like the whole goal was to make cyclic adenosine 3′,5′-monophosphate (cAMP). The β_3AR had just been discovered and was principally in the adipocyte, so now here was a cell with perhaps all three βARs.

Over time, it has become well established that βARs are the primary regulators of the catabolic recruitment of stored triglyceride breakdown in adipocytes. In brown adipocytes, they are also the prime drivers of the machinery of uncoupled mitochondrial respiration and adaptive nonshivering thermogenesis. Signaling from βARs to cAMP and protein kinase A (PKA) was considered the primary and singular biochemical pathway that controlled all of these processes. Over the years, like many areas of biomedical research, the picture has become more complicated, with many interconnected signaling mechanisms operating together, sometimes in curiously unexpected ways. Here, I review the history of our current understanding of the role of βARs and cyclic nucleotides in adipose tissue metabolism.

THE EARLY MOUSE MODELS OF OBESITY

During the early 1990s when our lab started studying βARs in adipose tissue, one of the major mouse models used in the obesity field was the *ob/ob* (obese) mouse. A related, but genetically distinct model, the *db/db* (diabetic) mouse, was also studied, but the latter more in the area of β-cell failure and diabetes. These mice were extensively characterized in the seminal publications of Doug Coleman and colleagues at the Jackson labs. In fact, Coleman predicted on the basis of physiological studies and parabiosis experiments (7–9) that the *ob* mutation led to the lack of a circulating factor and that the *db* mutation was the receptor for this factor. Later, in the subsequent elegant molecular work of Jeff Friedman and collaborators (10–12), this is exactly how it turned out, when molecular cloning of the loci revealed the full gene sequences and these mutations. We now know the *ob/ob* mouse as the leptin-deficient mouse and the *db/db* mouse as the leptin-receptor mutant mouse. Upon Colemans's passing, Friedman wrote an appreciative memorial of him (13).

REALIZING THERE WERE THREE βARs

Preceding the isolation and sequencing of the *ob* and *db* alleles, the *ob/ob* mouse was a major model in studies of adipose tissue and obesity, but it left investigators who were studying adipocytes in particular rather flummoxed. The data clearly showed some kind of deficit in adipocyte βAR signaling in these mice, as indicated by the blunted production of cAMP, a relative lack of lipolysis, and impaired nonshivering thermogenesis. However, no one could find anything wrong with the levels or activity of β_1AR and β_2AR (at this time, a third βAR was yet to be discovered) (14–17). Studies also probed a possible perturbation of the heterotrimeric G proteins in *ob/ob* adipocytes, given that βARs couple to G proteins and cAMP production, but these studies did not reveal them to be the cause. Some pharmacologists noted that there was something odd about the Schild

plots of β_1AR and β_2AR in adipose tissue preparations, which used established receptor subtype-selective antagonists (18). The results did not fit a clean profile for proportions of β_1AR and β_2AR. There was discussion about whether the lipid properties of the plasma membrane—something about the high amount of triglyceride in the cells or something else—was impacting the behavior of β_1AR and β_2AR in adipocytes. Other compounds with β-adrenergic properties that were developed, such as BRL37344 from Beecham Research Labs (United Kingdom), also found the same discrepancies in that they were unable to clearly ascribe to the adipocytes whether the receptors were β_1AR or β_2AR. Thus, some referred to the theoretical possibility that one of these two receptors had special properties in this particular cell—or that there still might be a third βAR—and this theoretical receptor was referred to as the atypical βAR (19, 20). The reason that the β_3AR as a molecular entity had been missed pharmacologically was because the β-antagonists used at the time, including radioligands, were later discovered to bind weakly at best to β_3AR (21, 22). Lab groups like Bob Lefkowitz's, which cloned eight of the nine known adrenergic receptor genes (23) and where I had been a postdoctoral fellow, used mostly human complementary DNA libraries, and certainly none from adipose tissue. Lefkowitz has referred to the β_3AR as the one that got away.

Some of these newer β-adrenergic compounds, including BRL37344, were shown to have unusually potent lipolytic and thermogenic properties in rodent adipocytes (19, 24), and newer pharmacological studies (e.g., 25) further fanned the flames to understand the nature of the βARs in adipocytes. The molecular cloning from humans and rodents of a distinctly different G protein–coupled receptor (GPCR) most similar to the two known βARs (26–29) led to the recognition that this new receptor was abundant in rodent white and brown adipose tissue; additionally, because it mediated the effects of these novel atypical βAR agonists, the issue of the atypical adipose βAR was finally resolved. With this discovery of a third βAR subtype, we showed (30) that in the *ob/ob* mouse, the expression of this receptor subtype was essentially absent (**Figure 1**). In addition, expression of the β_1AR was also reduced by 80%, which had not been previously observed (30). In this study, the functional consequences of these deficits in expression were revealed in a series of detailed pharmacological experiments (30). Given that β_1AR and β_3AR were both significantly reduced, leaving only a modest amount of β_2AR in the tissue, and that norepinephrine released from nerve terminals innervating adipose tissue binds most potently to $\beta_1\text{AR} \geq \beta_3\text{AR} \gg \beta_2\text{AR}$ (31, 32), there was effectively very little stimulation of adipocyte βARs to stimulate lipolysis and thermogenesis. This also turned out to be the case for the other known mouse congenital obesity models at the time (33) as well as for the Zucker fatty rat (29). Importantly, similar decreases in the expression of the β_3AR and β_1AR have been shown to occur in diet-induced models of obesity in mice (34). In 1992, a far more selective β_3AR agonist was developed by American Cyanamid: CL316,243 (35) was shown to have powerful effects in rats and mice to reduce or prevent obesity (36), principally by decreasing fat mass as a consequence of increased lipolysis and thermogenesis.

DO β_3AR AND BROWN FAT PLAY A ROLE IN HUMAN OBESITY?

The realization that the β_3AR was the atypical βAR that possessed robust lipolysis and thermogenesis and reductions in adipose mass spurred interest in finding compounds to target this receptor to treat human obesity, with all major pharmaceutical companies at the time focused on this goal. Nevertheless, there was still much skepticism in the obesity research community about the value of the β_3AR as a drug target. Some studies reported that it was difficult to detect significant β_3AR in human adipocytes, certainly in comparison to the amount of β_3AR in rodent adipose tissue. Also at this time, the notion that humans had any brown fat beyond the neonatal period had been largely dismissed. I was often asked why I studied "that β_3AR and brown fat; they are only in rodents and have nothing to do with adult human physiology." My reply was that humans with

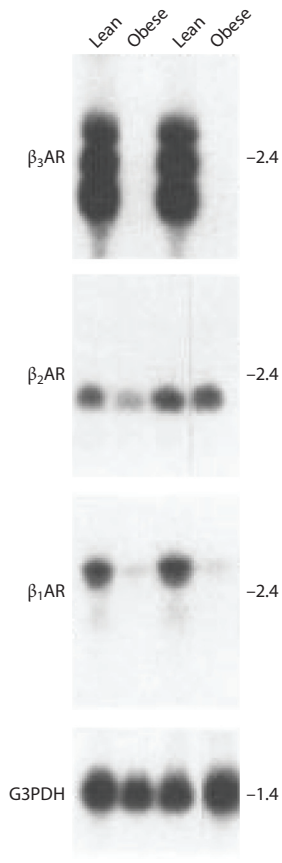


Figure 1

Northern blot of β_3AR, β_2AR, and β_1AR (adrenergic receptor) mRNA levels in white adipose tissue from 12- to 13-week-old genetically lean (+/+) and obese (*ob/ob*) mice. Transcript sizes in kilobases are indicated on the right side of each subpanel. Adapted with permission from Reference 30.

pheochromocytoma have a lot of brown fat around those tumors and they express robust amounts of the uncoupling protein 1 (UCP1). Therefore, these cells must exist in adult humans and there must be a way to recruit them in adults. It was soon discovered that compounds such as CL316,243 and BRL37344, which are very potent β_3AR agonists in rodents, are very weak agonists for the human β_3AR. With further screening, several newer compounds were found to be full or nearly full agonists for the human β_3AR, such as L755,507 from Merck (37) and mirabegron. However, despite encouraging results in nonhuman primates (38, 39) and acute effects on energy expenditure in obese humans (40), clinical trials of β_3AR agonists in obese humans lack efficacy (41–43), and interest in β_3AR as an obesity target faded. There are several points worth noting about this issue, and the reader is referred to primary research articles and reviews written by J.R.S. Arch, who has deep experience in this field and great perspective (e.g., see review in 44; many important published works on this topic by Arch can also be found).

With the rediscovery of brown fat in adult humans in 2009 based on ^{18}F-deoxyglucose uptake by positron emission tomography (FDG-PET) (45–48), interest in β_3AR as an obesity target resumed. Elegant human cold-exposure studies by Carpentier and Blondin and colleagues (49–53)

have provided detailed analyses of brown fat thermogenesis and energy substrate usage, illustrating how brown fat activation impacts metabolism. Mirabegron is a β_3AR agonist approved for the treatment of overactive bladder in humans (54). Its use in newer clinical studies of brown adipose tissue has met with mixed results. A study by Cypess et al. (55) found no effect of the recommended 50-mg dose on FDG uptake in subjects where one could detect cold-induced brown fat uptake, while a 200-mg dose was able to increase the FDG-PET signal. However, this large dose also caused elevated heart rate and blood pressure, likely due to spillover stimulation of β_1AR and β_2AR. Kern and colleagues (56), using the 50-mg dose of mirabegron, were able to observe beiging or browning of subcutaneous fat in obese subjects after a 10-day treatment. Moreover, they were able to show improvements in glucose parameters in obese humans (57). Additional studies using human adipocyte cultures obtained from postmortem superficial neck as well as supraclavicular adipose tissues present supportive evidence of functional activity of the human β_3AR on lipolysis and mitochondrial respiration, including increased expression of UCP1 in response to in vitro mirabegron treatment (58). These studies provide support for this therapeutic avenue, and yet other recent studies question a significant role for β_3AR and propose that β_1AR (59) or β_2AR (60) are the primary brown adipocyte signaling drivers responding to catecholamines in humans. An interesting article by Cypess et al. (61) reported that when using ephedrine as a sympathetic stimulant, it was unable to elicit FDG uptake in the manner that occurs in response to cold temperature. Does this mean the drug regimen was not optimal, or are there other biological pathways and events occurring with cold exposure that we do not yet understand? Observations by us and others that cold exposure produces changes in other hormones and receptors that also regulate adipocyte metabolism and are impacted by cold temperature might be part of this observed effect (62–65). In summary, there is clearly more work to be done to understand the role of the individual βAR subtypes and other signaling pathways in brown/beige fat biology.

WHY DO ADIPOCYTES EXPRESS THREE βARs WHEN THE SECOND MESSENGER IN EACH CASE IS cAMP?

That was the question that propelled me to study the βARs in adipocytes. While the original question has never been answered, along the way we and others in the field have learned a lot about signaling downstream of these receptors in adipocytes. As discussed elsewhere (66), each of the three βARs seems to have a distinct temporal and architectural means by which they engage other proteins involved in the signaling cascade. If we also take into consideration that the βARs may reside in distinct cellular compartments and interact with unique sets of proteins that are necessary for proper facilitation of their intracellular signaling networks, there is still much to be learned about this very fundamental question. For example, in the GPCR world, there is an extensive literature on the signaling events that occur upon ligand binding. In many if not most cases (β_3AR being an exception here), the receptor becomes phosphorylated on several intracellular regions by GPCR kinases (GRKs), which then serve as a docking platform for the binding of β-arrestins. In the earliest studies that defined these events, the consequence was viewed to be a mechanism for desensitization. It is now appreciated that a host of other adaptors and signaling molecules collaborate with the β-arrestins, leading to a wide range of other signal transduction pathways being triggered (for reviews about these arrestins, see 67, 68). During the early period of such studies, with my former postdoctoral mentor's lab just a couple of floors above me at Duke University, we wondered whether any of these new pathways might be occurring for the adipocyte βARs. An observation that was somewhat confusing was why activation of β_3AR seems to be such a powerful stimulus for driving brown adipocyte activation for thermogenesis pathways, when despite such a large content of β_3AR in rodent adipocytes, the net production of cAMP from adipocyte

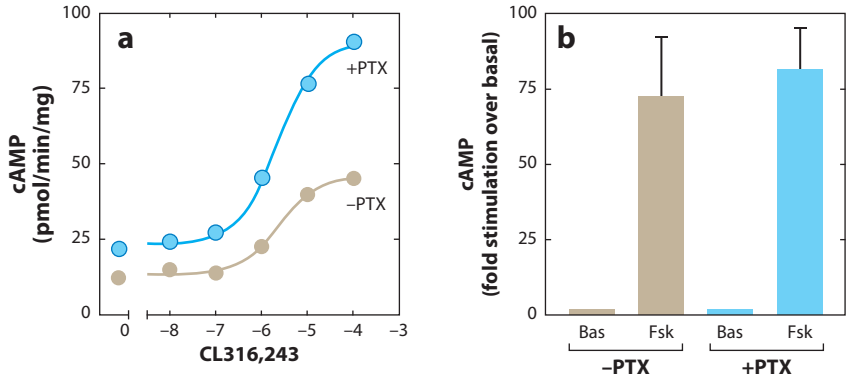


Figure 2

β_3-adrenergic receptor (β_3AR) agonist stimulation of cAMP production in 3T3-F442A adipocytes. (*a*) Dose-response curve for stimulation of cyclic adenosine 3′,5′-monophosphate (cAMP). Membranes of untreated or pertussis-toxin (PTX)-pretreated adipocytes were prepared and incubated with increasing agonist concentrations. cAMP production was measured by radioimmunoassay using polyclonal antisera to iodinated cAMP. (*b*) Basal (Bas) and forskolin (Fsk)-stimulated cAMP in untreated and PTX-pretreated membranes. Figure adapted from Reference 69 (CC BY 4.0).

plasma membranes was relatively modest (30). Through a series of studies we uncovered that β_3AR can interchangeably couple to both $G_{\alpha s}$ and $G_{\alpha i}$ (69) (**Figure 2**). We also discovered that, unlike other GPCRs, such as the β_2AR that activates the mitogen-activated protein kinase (MAPK) extracellular signal–regulated kinase (ERK), or MEK/ERK, pathway through the processive steps of phosphorylation of the receptor and interaction with β-arrestins (70), the intracellular domains of β_3AR (particularly the third intracellular loop and C terminus) contain the sequence motifs necessary for Src binding and ERK activation without the involvement of these other steps (71) (**Figure 3**). Functionally, we found that the ERK pathway appears to contribute to a modest proportion of β_3AR-stimulated lipolysis (72). There is also a report that phosphorylation of β_3AR by ERK appears to play a role in ERK-mediated lipolysis (73). Whether the β_3AR-activated ERK retroactively phosphorylates the receptor, whether the MEK/ERK pathway is activated by some other pathway, or both is not clear.

In addition to the role of GRKs and β-arrestins, the important concept of biased agonism is a fascinating aspect of GPCR biology, wherein ligands that bind to the same receptor can elicit largely G protein signaling with limited β-arrestin action, while other ligands at the same receptor trigger the reverse (for other reviews and more about these receptor signaling events, see 74, 75). Regarding the βARs in adipocytes (and the αARs), the field has just begun to scratch the surface of how these signaling modes may modulate the effects of catecholamines in adipocytes. There is certainly still much to investigate here and likely important discoveries to be made that might aid in translational work to target obesity and metabolic disease in humans.

MORE KINASES DOWNSTREAM OF βARs AND PROTEIN KINASE A IN ADIPOCYTES

While investigating the ability of β_3AR to activate the ERK arm of the MAPK pathway, serendipity shined upon us. A senior postdoctoral fellow in my lab, Wenhong Cao, told me one day that Cell Signaling Technology (Danvers, Massachusetts) had this little starter kit with the phospho- and total MAPK antibodies for ERK, c-Jun N-terminal kinase (JNK), and p38, which would allow

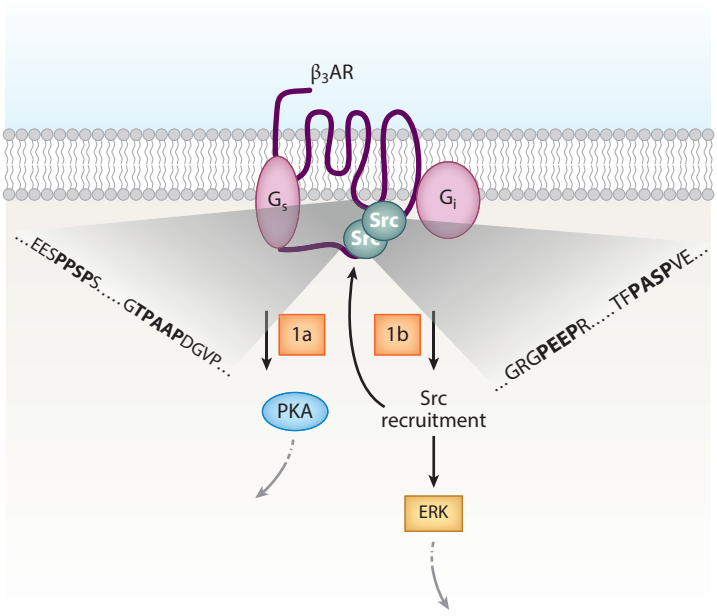


Figure 3

A unique dual signaling mechanism by β_3-adrenergic receptor (β_3AR). The receptor can couple to both G_s and G_i to activate protein kinase A (PKA) (step 1a) and extracellular signal–regulated kinase (ERK) (step 1b). The gray dotted arrows point to subsequent downstream cellular effects. Adapted from Reference 66 (CC BY-NC 3.0).

us to buy a small amount of the ERK antibodies and get the other ones along with it. This was mostly an exercise in "What if?" Not long after that, people in the lab came to me and said "Hey, p38 MAPK seems to get activated downstream of isoproterenol in 3T3-L1 white adipocytes and HIB-1B brown adipocytes!" That was somewhat unexpected, so what might it affect? There are four p38 MAPK subtypes that are the products of different genes (76), and there are selective small-molecule inhibitors for p38α and p38β MAPK (77), which at least gave us a tool to begin investigating the role of βAR-p38 MAPK signaling. Using these inhibitors as a starting point, we asked whether inhibiting p38 MAPK would affect lipolysis: The answer was no. So, what else should we look at? What about UCP1 expression in brown adipocytes, because that is another signature function downstream of βAR signaling? To our surprise, the p38α/β MAPK inhibitors completely blocked the ability of isoproterenol to increase UCP1 expression in a model brown adipocyte line, and we also determined that this pathway required the cAMP/PKA pathway (78). This initial study was followed up with a series of more extensive in vitro and in vivo studies using acute small interfering RNA (siRNA) knockdown strategies coupled with inhibitors and overexpression to address the signaling cascade and the physiological role of this PKA to p38α MAPK in the thermogenic gene expression pathway (79, 80) (**Figure 4**). In brown adipocytes, the PKA to p38 MAPK pathway also leads to p38-dependent phosphorylation of peroxisome proliferator-activated receptor-γ coactivator-1α (PGC1α) (79), which had been shown in skeletal muscle to drive its transcriptional coactivator function (81). It is now apparent that other hormones or neurotransmitters that promote brown adipocyte metabolism and thermogenesis also engage p38 MAPK (e.g., 62, 82–84). Another element we and our colleagues discovered was the important role of the scaffold protein p62 (also called sequestosome-1) as a critical chaperone to deliver p38

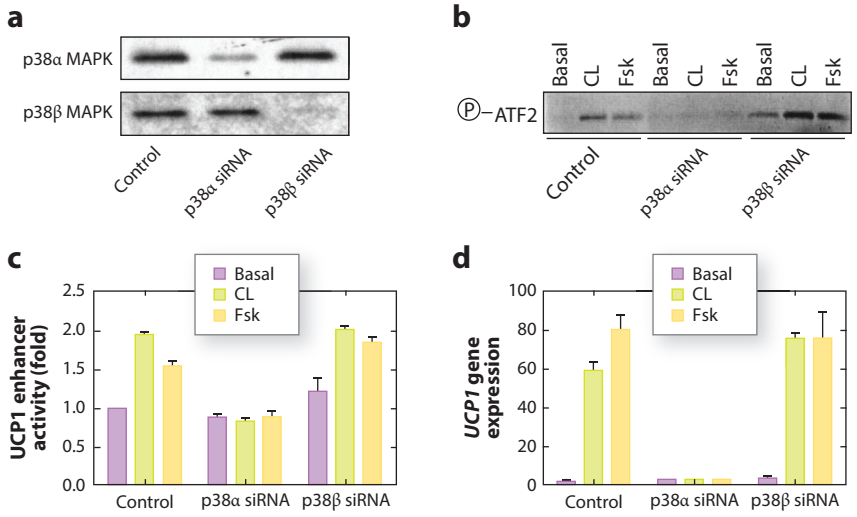


Figure 4

p38 MAP kinase mediates the βAR and cAMP activation of the *UCP1* gene. HIB-1B cells were transfected with siRNA targeting of p38α and p38β MAPK. (*a*) Expression of the kinases by Western blot. (*b*) Kinase activity using GST-ATF2 as a substrate. The circle containing a P indicates detection of phosphorylated ATF2. (*c*) HIB-1B cells were transfected with siRNAs targeting p38α and p38β MAPK, followed 12 h later with the β_3AR, UCP1 enhancer, and β-actin-luciferase plasmids. (*d*) RNA was extracted, and the measurement of UCP1 was evaluated by real-time PCR using a TaqMan probe. Figure adapted from Reference 80. Abbreviations: βAR, β-adrenergic receptor; β_3AR, β_3-adrenergic receptor; cAMP, cyclic adenosine 3′,5′-monophosphate; CAT, chloramphenicol acetyl transferase; CL, CL316,243; Fsk, forskolin; GST-ATF2, glutathione *S*-transferase-activating transcription factor 2; HIB-1B, hibernoma-1B cell line; MAP, mitogen-activated protein; PCR, polymerase chain reaction; siRNA, small interfering RNA; UCP1, uncoupling protein 1.

MAPK-phosphorylated ATF2 (85, 86) to its transcription factor binding sites at the *Ucp1* and *Pgc1α* genes, as we previously showed (79, 80). Genetic deletion of p38α in mouse adipose tissue has been done and led to some conflicting findings (87, 88). These reports showed that in the chronic absence of p38α MAPK from the adipocyte, it appears that p38δ MAPK becomes the responsive kinase, and these mice were shown to have more browning of white fat. Small-molecule inhibitors and gene knockouts that occur early in the development of a tissue can both have their shortcomings. Given the well-known capacity in genetic studies for compensatory gene expression (reviewed in 89, 90), perhaps additional work of a more acute nature, such as a constitutive and inducible knockout of p38δ in adipocytes, will answer the question, and inducible deletion of p38α in adult animals along with prompt examination of the effects would provide clarity. Although these details are of interest to the signal transduction mavens, discovery of the involvement of p38 and its requirement in adipocyte physiology for thermogenesis is the main take-home message.

UNCONVENTIONAL ACTIVATION OF MECHANISTIC TARGET OF RAPAMYCIN COMPLEX 1

Just as the link between cAMP/PKA and p38 MAPK in the adipocyte was an accidental observation, so too was the more recent discovery that PKA can also activate the rapamycin-sensitive mTOR complex 1 (mTORC1) in white and brown adipocytes (91). In this case, however, this seemed even more bizarre: The mTOR pathway is best known as being driven by growth factors and insulin, and in adipocytes, the insulin signaling pathway generally opposes catecholamine

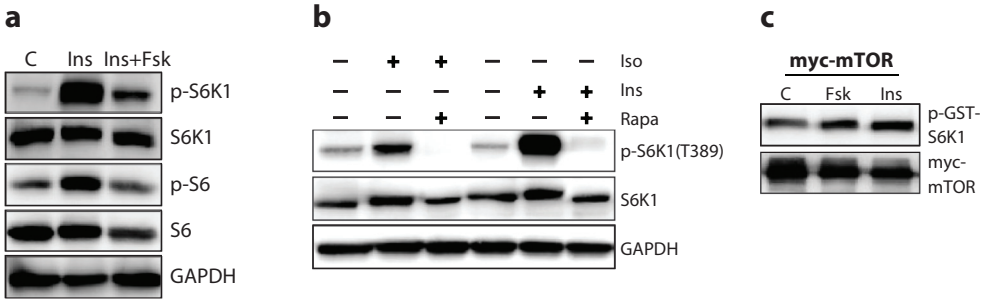


Figure 5

Insulin and βAR/cAMP signaling can activate mTORC1 activity in adipocytes. (*a*) 3T3-L1 adipocytes maintained in DMEM without FBS overnight, treated with insulin (Ins; 10 nM) alone or with forskolin (Fsk; 20 μM) 30 min prior to 30 min Ins (10 nM) treatment. Phosphorylated S6K1 and S6 and their respective total proteins were measured by Western blotting. GAPDH served as the loading control. (*b*) 3T3-L1 adipocytes were pretreated or not with rapamycin (Rapa; 100 nM) for 30 min prior to the addition of Iso (1 μM) or Ins (10 nM) for 1 h; respective total proteins were measured by Western blotting with GAPDH as the loading control. (*c*) Both Fsk and Ins increase mTOR kinase activity in vitro. 293T cells were transfected with 2 μg/well plasmid of myc-mTOR WT in 6-well plates. After 24 h, cells were treated with either Fsk (10 μM) or Ins (100 nM) for 30 min, and the remainder of the kinase assay was performed exactly as described. Adapted with permission from Reference 91. Abbreviations: βAR, β-adrenergic receptor; cAMP, cyclic adenosine 3′,5′-monophosphate; DMEM, Dulbecco's Modified Eagle Medium; FBS, fetal bovine serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Iso, isoproterenol; mTORC1, mechanistic target of rapamycin complex 1; WT, wild-type.

action. In classic work by John Lawrence, who worked on the mTOR pathway in 3T3-L1 adipocytes and made many seminal observations, his lab showed that insulin signaling increased the activity of the mTOR kinase, while the cAMP pathway blunted or blocked the insulin effect on mTOR (92). A replication of this result from our lab is shown in **Figure 5*a***. However, while this observation is certainly true, it turns out that the cAMP/PKA pathway itself has its own ability to activate mTOR, also in a rapamycin-sensitive manner (**Figure 5*b***). Conducting an old-fashioned mTOR kinase assay (93) also indicated that both isoproterenol (Iso) and insulin led to activity (**Figure 5*c***). When the lab first showed Western blots of S6K1 phosphorylation in 3T3-L1 adipocytes, and that cells treated with the βAR agonist Iso also activated S6K1, it was difficult to believe. The result had to be impossible based on Lawrence's work, and perhaps the insulin-stimulated and Iso-stimulated samples had been mixed up. After a few more experiments, it was impossible to ignore this finding. Indeed, 10 years of working on this topic finally resulted in our publication (91) demonstrating that PKA directly phosphorylates mTOR [three sites: serine (Ser)1276, Ser1288, and Ser2112] and Raptor (one site: Ser791), and that blockade of mTORC1 by either rapamycin or adipocyte deletion of Raptor impaired the ability of a β_3AR agonist or cold exposure to promote the browning of the subcutaneous adipose depots (**Figure 6**). In the mice with an adipoQ-Cre-mediated deletion of Raptor, even in the interscapular brown fat, the amounts of mitochondria and UCP1 were significantly reduced. By generating point mutations of the single PKA site in Raptor [Ser791Ala (alanine) or Ser791Asp (aspartic acid)], we could also show that activation of mTORC1 and S6K1 by insulin with either of these Raptor mutations was unaffected, but in the Ser791Ala mutant, PKA phosphorylation of this site was now lost. In contrast, in brown adipocytes engineered to express the Ser791Asp mutation, which becomes a phospho-mimetic, a host of genes for mitochondria and fatty acid oxidation are increased—even in the absence of βAR agonist. On the other hand, another publication shows that in cell culture, PKA can phosphorylate Raptor on Ser791, but in this case, the authors conclude that this modification results in inhibition

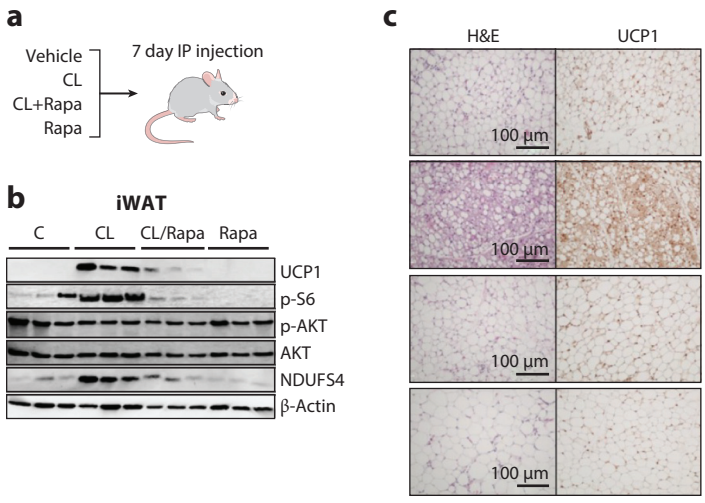


Figure 6

mTORC1 signaling participates in adipose browning induced by β_3AR activation. (*a*) Male mice were pretreated or not with rapamycin (Rapa; 2.5 mg/kg BW) for one day, followed by either vehicle control, CL316,243 (CL; 1 mg/kg BW/day), CL with Rapa, or Rapa alone daily treatment for 7 days. (*b*) iWAT (30 μg protein) analyzed by Western blotting for p-S6, p-AKT (S^{473}) and total AKT, mitochondrial UCP1, mitochondrial complex I component NDUFS4, and internal loading control β-actin. (*c*) Histochemical staining by H&E (*left side of panel*) and UCP1 antisera (*right side of panel*) from iWAT; sections are 20× magnification. Adapted with permission from Reference 91. Abbreviations: β_3AR, β_3-adrenergic receptor; BW, body weight; CL, CL316,243; IP, intraperitoneal; iWAT, inguinal white adipose tissue; mTORC1, mechanistic target of rapamycin complex 1; NDUFS4, NADH:ubiquinone oxidoreductase subunit S4; UCP1, uncoupling protein 1.

of mTORC1 in response to growth factors (94). This is entirely consistent with the original data of Lawrence and with our own findings. The response detected is based on what the prevailing stimuli are that are being examined, and when studies are performed in cell culture conditions loaded with serum and growth factors it is easy to miss the activating effects of PKA on mTORC1.

On reflection, while the thermogenic and energy-expending action of brown/beige adipocytes is a catabolic process, the browning of white adipocytes and the expansion of the interscapular depots to handle this demand for heat production are in themselves anabolic processes: the building of more mitochondria, more cells, and more fatty acid synthesizing capability. Thus, there are many questions to explore about this PKA-activated mTORC1: What are the downstream substrates of mTORC1 activated by β-agonists versus insulin? What is the composition of the mTORC1 complex with these bulky phosphate moieties on mTOR and Raptor? How broadly and in what different cell types does this pathway operate? This last question arises because, in a number of different cell types and other agonists that activate the cAMP pathway, one is led to consider that this βAR to PKA to mTORC1 pathway may function not only in adipocytes but in many other cell types. It will be important to explore these questions with the appropriately engineered animal models.

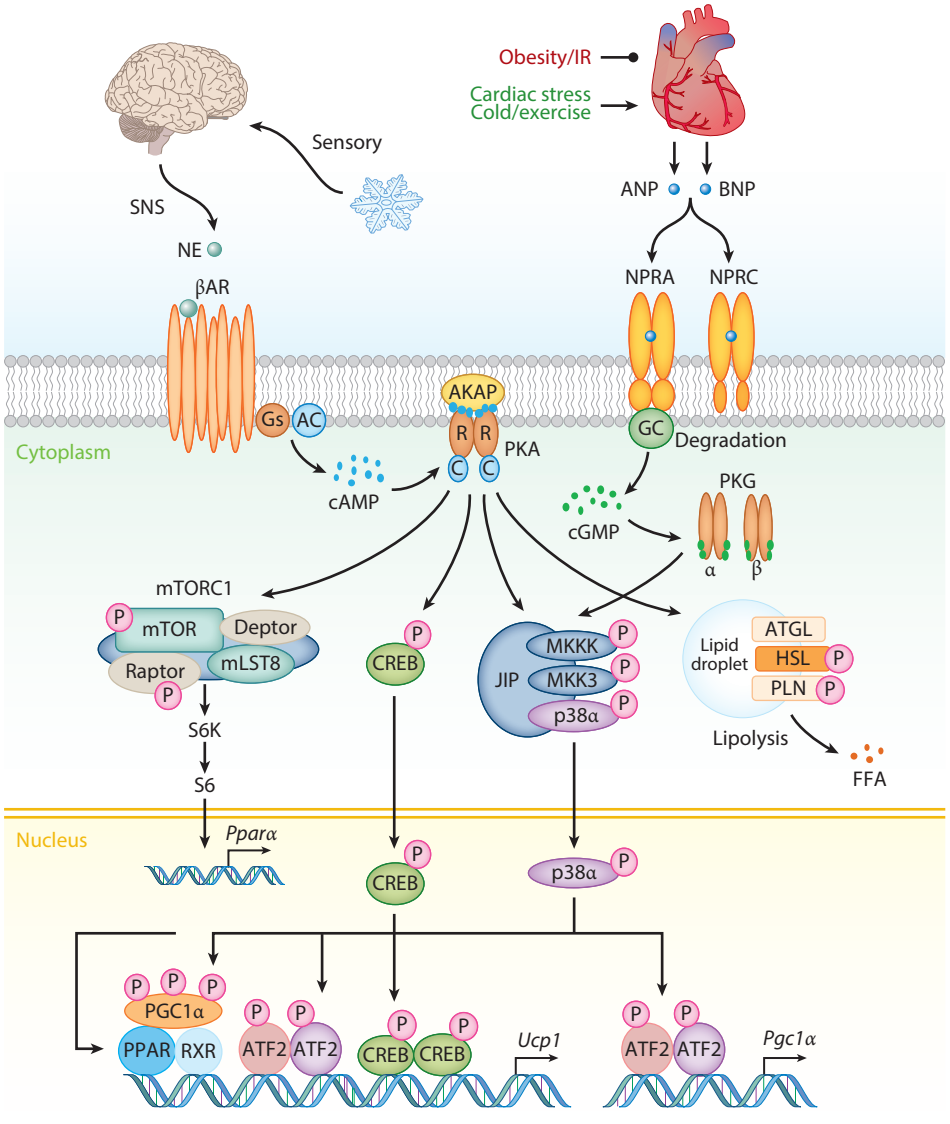
MORE THAN cAMP AND PROTEIN KINASE A

Although the focus of this review has been on the βARs in adipocytes and adipose tissue, the cGMP signaling pathway is analogous to the cAMP pathway. Cyclic guanosine 3′,5′-monophosphate (cGMP) that is produced by, for example, the cardiac natriuretic peptides, activates protein

kinase G (PKG), leading to the same thermogenic and browning functions of the PKA pathway (62) and which parenthetically also activates the p38 MAPK mechanism downstream of PKG. In a similar vein, we recently reported that natriuretic peptide signaling also leads to the activation of mTORC1 and adipose browning (95). **Figure 7** presents a final and rather busy summary of what is going on at the level of signal transduction in the adipocyte—at least so far as we currently know it.

CLOSING THOUGHTS

Why are all these pathways being activated in response to a single stimulus? One can envision specificity in signal transduction like it is a safe deposit box: One key alone does not open the box. The number of keys needed for any given response along with the temporal nature of the



(*Caption appears on following page*)

Figure 7 (*Figure appears on preceding page*)

An orchestra of signaling pathways controlling adipocyte metabolism. Cold and βAR agonist stimulation of adipocytes activate the cAMP/PKA pathway, which increases triglyceride lipolysis. The cAMP/PKA pathway also recruits the p38 MAP kinase and mTORC1 pathways. These three kinase nodes contribute to the overall lipolytic and thermogenic activities of the adipocyte by controlling the location and activity of transcription factors and their target genes, two of which are shown here: *Ucp1* and *Pgc1α*. In a parallel manner, the cardiac natriuretic peptides—released from atrial storage sites in response to elevations in blood pressure (including in response to cold)—activate their guanylyl cyclase coupled receptor NPRA, leading to cGMP/PKG activation. The same downstream kinase cascades are activated, resulting in lipolysis and thermogenesis. The NP clearance receptor, NPRC, exerts a moderating effect on NPRA signaling by removing NPs and degrading them. Abbreviations: AC, adenylyl cyclase; AKAP, A-kinase anchoring protein; ANP, atrial natriuretic peptide; ATF2, activating transcription factor 2; ATGL, adipose triglyceride lipase; βAR, β-adrenergic receptor; BNP, B-type natriuretic peptide; C, catalytic subunit; cAMP, cyclic adenosine 3′,5′-monophosphate; cGMP, cyclic guanosine 3′,5′-monophosphate; CREB, cAMP response element-binding protein; Deptor, DEP domain-containing mTOR-interacting protein; FFA, free fatty acid; GC, guanylyl cyclase domain; Gs, stimulating guanine nucleotide exchange protein; HSL, hormone-sensitive lipase; IR, insulin resistance; JIP, Jun kinase-interacting protein; MKK3, mitogen-activated protein kinase 3; MKKK, mitogen-activated protein kinase kinase kinase; mLST8, mammalian lethal with SEC13 protein 8; mTOR, mechanistic target of rapamycin; mTORC1, mTOR complex 1; NE, norepinephrine; NP, natriuretic peptide; NPRA, NP receptor A; NPRC, NP receptor C; P, phosphorylation; p38α, mitogen-activated protein kinase 14; PGC1α, peroxisome proliferator-activated receptor-γ coactivator-1α; PKA, protein kinase A; PKG, protein kinase G; PLN, perilipin; PPAR, peroxisome proliferator-activated receptor; *Pparα*, peroxisome proliferator-activated receptor α; R, regulatory subunit; Raptor, regulatory-associated protein of mTOR; RXR, retinoid X receptor; S6, ribosomal protein S6; S6K, S6 kinase; SNS, sympathetic nervous system; *Ucp1*, uncoupling protein 1. Figure created by Dr. Fubiao Shi.

stimulus will dictate whether the box gets opened. In the case of nonshivering thermogenesis in brown/beige adipocytes, it seems that the safe deposit box requires a number of keys, as engaging in ATP-consuming uncoupled respiration is not something to do accidently: This box has several keys, all of which have been turned at the same time to access the money! With the continued excitement over the potential of modest negative energy expenditure in UCP1-expressing adipocytes, and a caloric deficit equivalent to one candy bar per day, one could lose (or at least not gain) about 10 pounds or more per year. Nevertheless, there will always be a strong element of healthy eating and exercise in our human lifestyles in order to manage body fat percentage as well as skeletal muscle fitness that will be beneficial for healthy living and aging. In summary, although adipose tissue has been studied for centuries, it seems that the more we uncover, the more there is for us to discover.

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