Cell Research (2010) 20:1282-1284.

www.nature.com/cr

RESEARCH HIGHLIGHT

Hot stuff: thyroid hormones and AMPK

D Grahame Hardie[1]

[1]*College of Life Sciences, University of Dundee, Dow Street, Dundee, DD1 5EH, Scotland, UK*
Cell Research (2010) **20**:1282-1284. doi:10.1038/cr.2010.153; published online 9 November 2010

Every high school biology student is taught that thyroid hormones increase the metabolic rate. This conclusion mainly arose from the effects of hyperthyroidism, the clinical condition characterized by excessive production of the hormones. Symptoms include weight loss despite increased appetite, tremors, cardiac palpitations, irritability, intolerance to heat and increased perspiration. Although understanding of how thyroid hormones increase metabolic rate at the molecular level has been elusive, a recent paper by Antonio Vidal-Puig and colleagues in *Nature Medicine* [1] provides important new insights.

The thyroid gland produces mainly thyroxine (T4), but other tissues deiodinate this to the more potent hormone, tri-iodothyronine (T3). T3 binds to thyroid receptors (TRα, TRβ), members of the nuclear receptor family that act as hormone-activated transcription factors in the form of heterodimers with the retinoid X receptor. These receptors are widely expressed and it has generally been assumed that thyroid hormones exert their effects mainly on peripheral tissues. Various suggestions have been made as to how peripheral action of thyroid hormones might activate energy expenditure, including futile cycling of Na^+ across the plasma membrane, or of Ca^{2+} between the cytoplasm and the sarcoplasmic reticulum in muscle [2]. However, none of these peripheral mechanisms have been fully substantiated. T3 is also known to exert indirect effects via increased activity of the sympathetic nervous system, and many of the signs and symptoms of hyperthyroidism, including cardiac palpitations, hand tremors and sweating, may be attributed to this. Vidal-Puig and colleagues [1] now provide evidence that increases in metabolic rate induced by thyroid hormones involve inhibition of AMP-activated protein kinase (AMPK) in the hypothalamus (the key brain region that is known to regulate appetite, body temperature and circadian rhythms), thus triggering heat production in brown fat tissue via the sympathetic nervous system.

AMPK occurs ubiquitously in eukaryotic cells as heterotrimeric complexes comprising catalytic α subunits and regulatory β and γ subunits [3]. At the cellular level it acts as an energy sensor activated by metabolic stress, which is detected by monitoring the ratios of adenine nucleotides. Genetic studies show that in the yeast *Saccharomyces cerevisiae*, and other lower eukaryotes such as *Caenorhabditis elegans,* it is required for responses to starvation, so that its ancestral role may have been that of a sensor of carbon nutrients [3]. It has been previously shown that agents that activate AMPK in the hypothalamus in rodents, such as ghrelin or the pharmacological activator 5-aminoimidazole-4-carboxamide riboside (AICAR), increase food intake while, conversely, those that inhibit AMPK, including leptin, reduce food intake [4]. There is already some evidence that AMPK regulates the activity of the sympathetic nervous system. First, mice with a global knockout of AMPK-α2 (one of two catalytic subunit isoforms) have increased urinary catecholamine levels, and exhibit a glucose intolerance that is reversed by the α-adrenergic antagonist, phentolamine [5]. Second, mice injected with leptin into the hypothalamus show a local inhibition of AMPK [6] that is followed by a slower activation of AMPK in soleus muscle [7]. The muscle effect is sensitive to denervation and blocked by phentolamine, indicating that it is mediated by the sympathetic nervous system [7].

In the new study, Vidal-Puig and coworkers [1] initially made rats hyperthyroid by daily subcutaneous injections of T4. As expected, this caused elevation of plasma T3, and the rats gained less weight than controls, despite eating more. The rats also had increased mass of brown fat, a tissue that can increase energy expenditure via heat production from uncoupled mitochondria when stimulated by sympathetic nerves (non-shivering thermogenesis). Although brown fat was once thought to be absent in adult humans, this view has recently been revised [8, 9]. The Vidal-Puig team monitored various markers of AMPK signaling in the hypothalamus, and provided evidence that T3 down-regulates AMPK in this location. Thus, phosphorylation of AMPK at the activating site

Correspondence: D Grahame Hardie
Tel: +44-1382-384253
E-mail: d.g.hardie@dundee.ac.uk

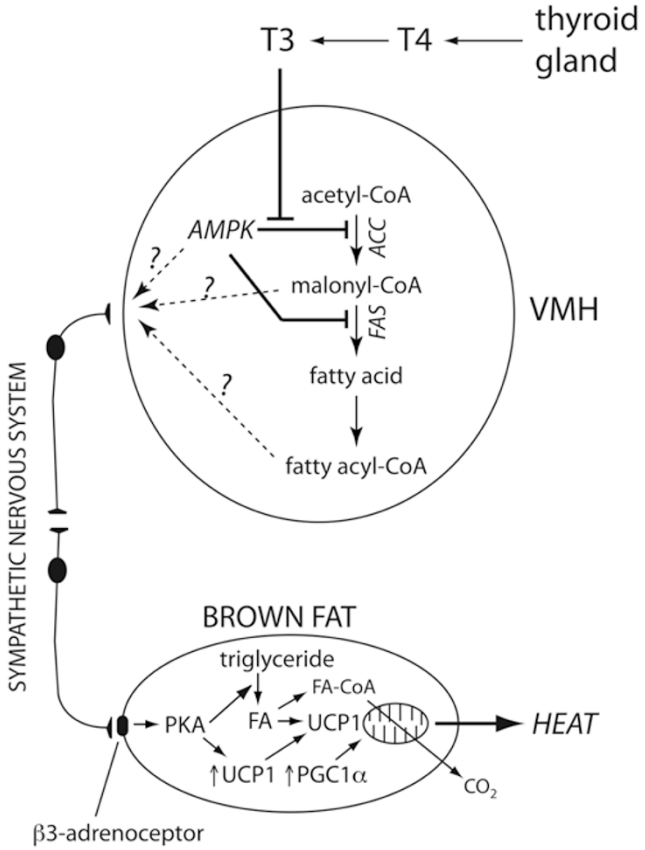

Figure 1 Proposed mechanism by which the thyroid hormone T3 promotes energy expenditure. T3 inhibits AMPK in cells of the ventromedial hypothalamus (VMH) by unknown mechanisms. This causes firing of action potentials in the sympathetic nervous system, either directly via AMPK, or indirectly via lipid metabolites whose metabolism is regulated by AMPK. The sympathetic nervous system acts via β3-adrenoceptors on brown adipose tissue cells, triggering (via cyclic AMP-dependent protein kinase, PKA) lipolysis, fatty acid oxidation and activation of protein leak by UCP1 in the short term, and up-regulation of UCP1 expression and mitochondrial biogenesis (via PGC-1α) in the longer term. The end result is increased heat production via the futile cycle of protons across the inner mitochondrial enzyme that is driven by the respiratory chain and UCP1.

(Thr172) and of its downstream target acetyl-CoA carboxylase (ACC, which is inactivated by phosphorylation) were both reduced in the hyperthyroid rats. The expression of the α1, but not the α2, subunit of AMPK was also reduced, while expression of fatty acid synthase, a lipogenic enzyme whose expression is down-regulated by AMPK in other tissues [10], was increased.

Next, T3 was administered locally to the hypothalamus by intracerebroventricular injection, which does not increase plasma T3. A key finding was that the effects were essentially the same as those when T4 was given peripherally. The rats gained less weight than controls, and the effects on AMPK signaling in the hypothalamus were very similar. Hypothalamic T3 injection also increased expression of mRNAs encoding thermogenic markers in brown fat, including the uncoupling protein UCP1. Although brown fat thermogenesis was not directly measured, it could certainly account for the reduced weight gain of the animals if it increased. These peripheral effects of central T3 administration appeared to be due to activation of the sympathetic nervous system, because there was increased electrical activity of sympathetic nerves supplying brown fat, while the expression of thermogenic markers was blocked by an antagonist of β3-adrenoceptors, which mediate effects of sympathetic nerves on brown fat.

To pinpoint the precise location of the effects, they also administered T3 by direct stereotaxic injection into the ventromedial hypothalamus (VMH). Like the intracerebroventricular injection, this reduced phosphorylation of hypothalamic AMPK and ACC, and increased electrical activity in sympathetic nerves supplying brown fat. To confirm the role of AMPK, they administered the AMPK activator AICAR by intracerebroventricular injection, or an adenovirus encoding an activated AMPK mutant by direct injection into the VMH. In hyperthyroid rats this caused weight gain and reduced expression of thermogenic markers in brown fat. Conversely, delivery to the VMH in euthyroid rats of adenovirus encoding a dominant negative mutant of AMPK-α1 had the opposite effects, causing weight loss and increased expression of brown fat thermogenic markers, both effects being reversed by the β3-adrenoceptor antagonist.

These results provide good evidence that stimulation of the VMH by T3 leads to local inactivation of AMPK, and that this in turn increases the activity of the sympathetic nervous system. The latter would then increase whole body energy expenditure by stimulating thermogenesis in brown fat (Figure 1), while also potentially explaining some of the other signs and symptoms of hyperthyroidism. The study provides important new insights into the central actions of thyroid hormones. Two obvious questions remain: how does activation of the thyroid receptor in the VMH cause inhibition of AMPK, and how does

this lead to downstream effects on the sympathetic nervous system? With respect to the second question, the authors propose that inactivation of AMPK alters lipid metabolism in the VMH, and that it is a downstream lipid metabolite that propagates the signal. Indeed, they mention that they could measure various changes in lipid metabolites in the hypothalamus in response to hyperthyroidism, although these were not reported in detail. There is an extensive body of literature suggesting that intermediates of fatty acid metabolism, such as malonyl-CoA or long chain acyl-CoAs, might be signals involved in nutrient sensing in the hypothalamus, as well as the pancreatic β cell [11, 12]. However, much of the evidence for this is based on the use of pharmacological inhibitors, or over-expression or knockdown of enzymes, which could have complex secondary effects on other metabolites. There also appears to be no current consensus concerning the sensors that monitor these lipid metabolites. At this point a rather simpler hypothesis springs to mind. A common feature of almost all agents that regulate outputs from the hypothalamus, whether in terms of feeding behavior or regulation of the sympathetic nervous system, is modulation of AMPK. Is it not possible that AMPK directly mediates the downstream effects, and that changes in lipid metabolites like malonyl-CoA are merely markers of its activity? AMPK orthologues are involved in nutrient sensing in lower eukaryotes, and it seems plausible that this system has been coopted in mammals to act in the response to hormones and other factors that coordinate metabolism and energy balance at the whole body level. AMPK is exquisitely sensitive to metabolic changes within a particular cell due to sensing of adenine nucleotides, and it can also respond directly to some hormones via the upstream Ca^{2+}-CaMKK pathway [13]. There is also a precedent for AMPK modulating neuronal activity. In type 1 cells in the carotid body, AMPK is thought to monitor oxygen availability by sensing changes in their rate of metabolism [14]. Once activated by hypoxia, AMPK phosphorylates and inactivates the voltage-gated K^+ channel, BK_{Ca}, causing plasma membrane depolarization and consequent release of neurotransmitters that initiate action potentials in afferent fibers leading to the brain. The end result is increased breathing to alleviate the hypoxia [14]. Finally, there is already good evidence that AMPK is involved in glucose sensing in the hypothalamus. Pro-opiomelanocortin (POMC)-expressing neurones in the hypothalamus, as well as a proportion of Agouti-related protein (AgRP)-expressing neurones, became hyperpolarized and reduced their spike firing frequency when exposed to low glucose. However, these responses were lost when AMPK-α2 was knocked out in these specific neurons [15]. Thus, AMPK seems to be well placed to directly regulate the sympathetic nervous system in response to thyroid hormones and other agents that act on the hypothalamus.

References

1 Lopez M, Varela L, Vazquez MJ, *et al.* Hypothalamic AMPK and fatty acid metabolism mediate thyroid regulation of energy balance. *Nat Med* 2010; **16**:1001-1008.
2 Silva JE. Thermogenic mechanisms and their hormonal regulation. *Physiol Rev* 2006; **86**:435-464.
3 Hardie DG. AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy. *Nat Rev Mol Cell Biol* 2007; **8**:774-785.
4 Kahn BB, Alquier T, Carling D, Hardie DG. AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. *Cell Metab* 2005; **1**:15-25.
5 Viollet B, Andreelli F, Jorgensen SB, *et al.* The AMP-activated protein kinase α2 catalytic subunit controls whole-body insulin sensitivity. *J Clin Invest* 2003; **111**:91-98.
6 Minokoshi Y, Alquier T, Furukawa N, *et al.* AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. *Nature* 2004; **428**:569-574.
7 Minokoshi Y, Kim YB, Peroni OD, *et al.* Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. *Nature* 2002; **415**:339-343.
8 Cypess AM, Lehman S, Williams G, *et al.* Identification and importance of brown adipose tissue in adult humans. *N Engl J Med* 2009; **360**:1509-1517.
9 van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, *et al.* Cold-activated brown adipose tissue in healthy men. *N Engl J Med* 2009; **360**:1500-1508.
10 Foretz M, Carling D, Guichard C, Ferré P, Foufelle F. AMP-activated protein kinase Inhibits the glucose-activated expression of fatty acid synthase gene in rat hepatocytes. *J Biol Chem* 1998; **273**:14767-14771.
11 Ruderman N, Prentki M. AMP kinase and malonyl-CoA: targets for therapy of the metabolic syndrome. *Nat Rev Drug Discov* 2004; **3**:340-351.
12 Lam TK. Neuronal regulation of homeostasis by nutrient sensing. *Nat Med* 2010; **16**:392-395.
13 Stahmann N, Woods A, Carling D, Heller R. Thrombin activates AMP-activated protein kinase in endothelial cells via a pathway involving Ca2+/calmodulin-dependent protein kinase kinase beta. *Mol Cell Biol* 2006; **26**:5933-5945.
14 Evans AM, Hardie DG, Peers C, *et al.* Ion channel regulation by AMPK: the route of hypoxia-response coupling in the carotid body and pulmonary artery. *Ann N Y Acad Sci* 2009; **1177**:89-100.
15 Claret M, Smith MA, Batterham RL *et al.* AMPK is essential for energy homeostasis regulation and glucose sensing by POMC and AgRP neurons. *J Clin Invest* 2007; **117**:2325-2336.