Curbing mitochondrial appetite

By Lev Osherovich, Senior Writer

Several companies have tried and failed to develop obesity drugs that agonize mitochondrial uncoupling proteins, which let adipose cells generate heat by burning excess fat. A new study now suggests that one member of this protein family, uncoupling protein 2 (mitochondrial, proton carrier), plays a different role in the brain—scavenging reactive

oxygen species—and should actually be antagonized to suppress appetite.¹ Although companies and academics think blocking this protein may be easier than agonizing it, the challenge of getting compounds into the brain and mitochondria remains formidable.

A team from **Yale University**, led by Tamas Horvath, professor of comparative medicine, neurobiology, obstetrics and gynecology, and Sabrina Diano, assistant professor of obstetrics and gynecology, reported in *Nature* that uncoupling protein 2 (mitochondrial, proton carrier) (UCP2) sits downstream of a hotly pursued metabolic target in the obesity space: ghrelin.

“It’s a nice first-pass study to look at neuronal and biochemical cascades that happen upon ghrelin signaling,” said Peter DiStefano, CSO of **Elixir Pharmaceuticals Inc.** “It gives us new places to look for possible targets of ghrelin pathway signaling antagonists.”

Building on previous studies implicating UCP2 in glucose sensing^{2,3} and using a combination of mouse genetics and metabolic techniques,

Figure 1. Sensing energy levels. The mitochondrial protein uncoupling protein 2 (mitochondrial, proton carrier) (UCP2) could be a pivotal player in metabolic diseases such as obesity and diabetes.

In the pancreas, UCP2 senses energy levels and downregulates the secretion of insulin from β -cells. Diao *et al.* now show that UCP2 also downregulates glucagon secretion by α -cells and could thus be targeted to treat hypoglycemia in type 1 diabetes.⁹

In the brain, Andrews *et al.* show that UCP2 mediates the appetite-promoting effects of the peptide hormone ghrelin in the arcuate nucleus of the hypothalamus.¹ In neurons that produce neuropeptide Y (NPY) and agouti-related protein (AGRP), ghrelin stimulates fatty acid oxidation, which increases UCP2 activity. As a result, levels of reactive oxygen species fall, leading to NPY/AGRP neuron firing. This signal promotes feeding by inhibiting downstream neurons that otherwise would produce the satiety hormone proopiomelanocortin (POMC).

UCP2 can be inhibited by genipin, a compound from *Gardenia jasminoides* fruit that is used as a research reagent. A number of companies are targeting the ghrelin receptor, which is upstream from UCP2, as well as downstream POMC neuron activity.

Horvath and Diano examined how the enzyme affected a neuronal relay that governs appetite in response to ghrelin.

Ghrelin, a peptide hormone, is produced by the stomach and acts in several tissues of the CNS and periphery to elicit hunger.⁴ Among ghrelin's targets are the neuropeptide Y (NPY) and agouti-related protein (AGRP) neurons in the hypothalamus. NPY/AGRP neurons in turn inhibit another group of neurons that produce proopiomelanocortin (POMC), a multihormone polypeptide that suppresses appetite.

The team found that UCP2 knockout mice treated with ghrelin showed less NPY/AGRP neuron firing and a lower feeding response than ghrelin-treated wild-type controls.

The researchers determined that ghrelin signaling in NPY/AGRP neurons promotes the phosphorylation of AMP-activated protein kinase (AMPK; PRKAB1) and acetyl-coenzyme A carboxylase- α (ACACA; ACC). This shifts the cell's metabolism from burning glucose to burning fatty acids through β -oxidation, a process that generates high levels of reactive oxygen species (ROS).

Over time, high ROS levels are thought to shut down β -oxidation, choking off the energy supply and lowering neuronal activity (see **Figure 1, "Sensing energy levels"**).

"The function of UCP2 has been discussed very much in the last few years," said Diano, "but it was controversial what it does in the brain."

The team found that UCP2 keeps ROS levels in check, thus preserving NPY/AGRP neuron activity.

Indeed, UCP2 knockout mice injected with a cocktail of ROS-

scavenging compounds responded normally to ghrelin, whereas untreated knockout mice did not respond to the hormone.

Hungry for more

Diano thinks the interplay between UCP2 and ROS presents a new point of therapeutic intervention to control appetite. Inhibiting UCP2 could potentially increase ROS levels in the NPY/AGRP neurons and thus lower food intake, she noted.

Diano said experiments are under way to test the effect of timing of antioxidant ingestion on ghrelin-triggered feeding.

Companies developing modulators of ghrelin signaling and its downstream neuropeptide targets to treat metabolic disorders told *SciBX* that the Yale study opens up ghrelin's mechanistic black box. However, they think neuronal surface receptors involved in appetite are better targets because they're easier to reach.

"This is the first instance I am aware of that shows regulation of mitochondrial activity as an acute controller of neuronal activity," said Michael Cowley, CSO of **Orexigen Therapeutics Inc.**

Targeting fatty acid oxidation could be an "entirely new modality of regulation of neuronal activity," said Cowley, who previously collaborated with Horvath to study the connection between NPY/AGRP and POMC neurons.

Orexigen's Contrave, a formulation of naltrexone and bupropion, promotes POMC neuron activity and is in Phase III trials for obesity.

Cowley said the most useful finding of the paper is pinpointing the

"[The study] gives us new places to look for possible targets of ghrelin pathway signaling antagonists."

**—Peter DiStefano,
Elixir Pharmaceuticals Inc.**

Box 1. Now UCP, now you don't.

Tamas Horvath and Sabrina Diano of Yale University show in their *Nature* paper that inhibiting UCP2 in regions of the brain could be useful in curbing appetite.¹ However, researchers polled by *SciBX* agreed that targeting UCP2 therapeutically remains a tall order due to problems of tissue selectivity and the different roles the enzyme plays in different cell types.

A case in point is a study showing that UCP2 directly controls glucagon secretion by α -cells in pancreatic islets, rather than insulin secretion by β -cells as previously thought.⁹

The finding, published in the *Proceedings of the National Academy of Sciences*, suggests that inhibiting UCP2 could stimulate glucagon secretion in pancreatic α -cells and thus be useful for controlling hypoglycemia in type 1 diabetes.

Pancreatic islets contain several kinds of hormone-secreting cells, notably the insulin-producing β -cells and

the glucagon-producing α -cells. Insulin tells the liver to pack away sugar after a meal, whereas glucagon tells the body to release sugar into the blood between meals. Type 1 diabetes patients cannot regulate blood sugar and often suffer from hypoglycemia, which is currently treated with glucagon injections.

UCP2 has previously been explored as a target for type 2 diabetes based on studies showing that UCP2 knockout mice secreted more insulin than wild-type controls and were resistant to diabetes-like effects of a high-fat diet.¹⁰

The new study in *PNAS*, from a team led by Michael Wheeler, professor of medicine and physiology at the **University of Toronto**, examined UCP2 in isolated mouse α -cells and β -cells, as well as cultured α -cell and β -cell lines. In all cases, the protein was found predominantly in α -cells. Consistent with an α -cell role for UCP2, isolated islets from UCP2 knockout mice showed higher rates of ATP synthesis and basal glucagon secretion than wild-type controls.

However, islet cells from UCP2 knockout mice proved more susceptible to cell death induced by serum starvation and inflammatory cytokines than wild-type controls.

"UCP2 is expressed in many tissues throughout the body, but it seems to play different roles in each one," said Emma Allister, a postdoc at the University of Toronto who was one of the lead authors on the *PNAS* paper. "It's very controversial whether it has a positive or negative role in islets."

The next step is to make cell-specific knockouts or inhibitors of UCP2 to test whether its most relevant role in diabetes lies in α -cells, β -cells or elsewhere.

"It is imperative to study UCP2's role in individual cell types not only in the pancreas but also in the brain, where it also appears to play a role in glucose sensing and food intake," Allister said.

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neuronal site of action of ghrelin. This may be helpful in designing assays and drug screens to find better antagonists of ghrelin and other appetite-promoting hormones.

However, Elixir's DiStefano cautioned that the effects of blocking UCP2 might be different in the periphery, where ghrelin also plays a role in appetite.

"It's important to realize that AMPK is complicated and ghrelin has opposite effects in the brain and periphery," he said. "In the periphery, ghrelin seems to decrease AMPK activity," presumably inhibiting UCP2 and thus potentially increasing appetite.

As a result, it is hard to predict whether inhibiting UCP2 would have desirable effects on peripheral metabolism.

DiStefano also noted that it would be difficult to specifically modulate ROS levels in the NPY/AGRP neurons, as high ROS levels are generally considered bad for cellular health.

Elixir has ghrelin receptor antagonists in preclinical development for type 2 diabetes and possibly obesity, and the company's scientists have published several studies on the role of AMPK and lipid metabolism in appetite.^{5,6}

Carl Spana, president and CEO of **Palatin Technologies Inc.**, agreed that the study's conclusions about UCP2's neuronal role in ghrelin signaling may not translate to other tissues, because AMPK and UCP2 are present in multiple cell types. Thus, he said, targeting neurons downstream of NPY/AGRP is a better tactic for treating appetite disorders than tinkering with the inner workings of ghrelin signaling.

Spana suggested that AMPK and UCP2 activity could instead be useful markers for monitoring fatty acid metabolism in neurons.

Palatin and partner **AstraZeneca plc** are developing melanocortin 4 receptor (MC4R) agonists to treat obesity and metabolic disorders. MC4R is downstream of POMC.

Tried and tired

Despite the potential therapeutic strategies offered by the new understanding of UCP2, researchers agreed it remains a difficult target.

The only published specific inhibitor of UCP2 is genipin, a natural compound derived from the gardenia fruit. In mice, genipin stimulates insulin secretion in cultured pancreatic β -cells⁷ (see Box 1, "Now UCP, now you don't"). In 2006, researchers at **Harvard Medical School** filed a patent for the use of genipin and related compounds to treat diabetes.

Karoly Nikolich, CEO of neuroscience startup **Amnestix Inc.** and consulting professor of psychiatry at **Stanford University**, said the biggest obstacles to targeting UCP2 are its mitochondrial localization and a lack of good screening methods for drug discovery.

Nikolich was a founder of AGY Therapeutics Inc., a neuroscience company that identified a role for UCP2 in ischemia.⁸ In 2005, however, AGY folded and transferred its IP to **Pfizer Inc.** and **M's Science Corp.** The company's UCP2 project was not picked up.

AGY used cell culture and yeast-based screens to hunt for UCP2 modulators, but primary screen hits didn't translate to higher organisms.

"UCP2 screens are very, very lousy," agreed Tamas Bartfai, professor of neuropharmacology at **The Scripps Research Institute** and former head of CNS research at **Roche**. "Compounds need to cross the blood-brain barrier, the plasma membrane and then the mitochondrial membrane."

According to Bartfai, industry has tried to target UCPs for 18 years but has yet to find a sufficiently potent but nontoxic compound.

"If I had 50 metabolic targets, I would not pick this one," he said.

Millennium Pharmaceuticals Inc., now part of **Takeda Pharmaceutical Co. Ltd.**, obtained a patent for the use of UCP2 to treat obesity in 1998, but a drug development partnership for this target with Roche yielded only preclinical results. The companies terminated the partnership in May 2008, according to a Millennium spokesperson.

Horvath and Diano have filed for patents on techniques to manipulate ROS levels in the hypothalamus to regulate feeding behavior. The technology is available for licensing through Yale University.

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