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Physiology

# Orphan detectors of metabolism

Steven C. Hebert

There are myriad G-protein-coupled receptor proteins in living organisms, but the functions of many are unknown. Two of them are now shown to provide a link between metabolism and blood pressure.

One of the first things that biology students are taught is how cells liberate usable energy from food and oxygen. In eukaryotic cells (loosely speaking, those that have nuclei), much of this process of respiration occurs in double-membrane-bounded compartments known as mitochondria. A crucial stage in respiration is the tricarboxylic acid (TCA) cycle or Krebs cycle[1], into which are fed the products of the breakdown of sugars, fats and proteins, and out of which comes reducing power, used to generate the energy-storing molecule ATP. As testimony to the importance of this process, mutations in human genes encoding TCA-cycle enzymes have been associated with inherited cancers and severe generalized organ abnormalities[2]. It has been known for some time that TCA-cycle intermediates are also present outside cells, in the blood circulation, and that extracellular levels of these organic acids increase when tissues are damaged by low oxygen supplies or other 'insults'. On page 188 of this issue, He and colleagues[3] show, unexpectedly, that two such intermediates act as signalling molecules, linking the metabolism and injury of tissues with blood pressure.

Cells can detect and respond to a wide range of external signals by using receptor proteins on their surfaces. G-protein-coupled receptors (GPCRs) are the largest and most diverse group of such detectors; as their name suggests, they transmit extracellular information into cells by coupling to G proteins — common molecular switches — in the cytoplasm[4]. Although all GPCRs have similar structures, they show considerable diversity at the sequence level. However, on the basis of specific sequence motifs, they have been divided into three distinct families, each containing subfamilies that often have related stimuli (ligands)[5,6].

The number of GPCRs is growing continually: many new putative receptor genes have been identified in genome databases by computer-based screening for similar sequences. But for many of the newly discovered receptors, a physiologically relevant ligand is unknown[6–8]. These 'orphan' receptors are of considerable interest to biologists because they might define new ways in which cells can respond to their external environment, and to the pharmaceutical industry because they might provide new targets for drug development. Finding the relevant ligand for an orphan receptor can be a daunting task, however. One approach has been to use phylogenetic analyses — based on similarities in amino-acid sequence — to place newly identified receptors into one of the GPCR subfamilies, in an attempt to identify the ligand by the company the receptor keeps. But this is frequently not successful, and finding the correct ligand often requires a wide-ranging screening approach.

This was just the case for the orphan receptors GPR91 and GPR99. A phylogenetic approach had hinted that these proteins might bind nucleotides, given their membership in the P2 purinoceptor subfamily. He *et al.*[3] have now found, however, that nucleotides do not activate these receptors. Instead, the TCA-cycle intermediates α-ketoglutarate and succinate are physiologically relevant ligands for GPR99 and GRP91 respectively.

What might these metabolic detectors do? Kidneys express both GPR91 and GPR99 (refs 7, 8), and He *et al.* provide compelling evidence that, via GPR91, succinate stimulates the release of renin from the kidneys. This reveals the mechanism behind the earlier observation that succinate increases the release of renin from isolated kidney capillaries[9]. Renin is part of the renin–angiotensin system (RAS) — a series of enzymes and molecules that culminate in the production of the eight-amino-acid peptide angiotensin II. This molecule in turn acts on blood vessels, causing them to constrict and so raising blood pressure.

Physiologically speaking, this could all be very useful. Mitochondrial dysfunction, caused by, for instance, an imbalance between energy demand and the food and oxygen supply, increases the extracellular concentrations of TCA-cycle intermediates (Fig. 1). He and colleagues' findings show that these intermediates can then influence the RAS, possibly (among other effects) reducing blood flow in the kidneys. Kidneys receive more than their fair share of the cardiac output of blood (about 20%), so reductions in kidney blood flow provide a means of redirecting blood to other organs during crises.

On the other hand, the balance can also be tipped towards disease: He *et al.* found that treating mice with succinate led to high

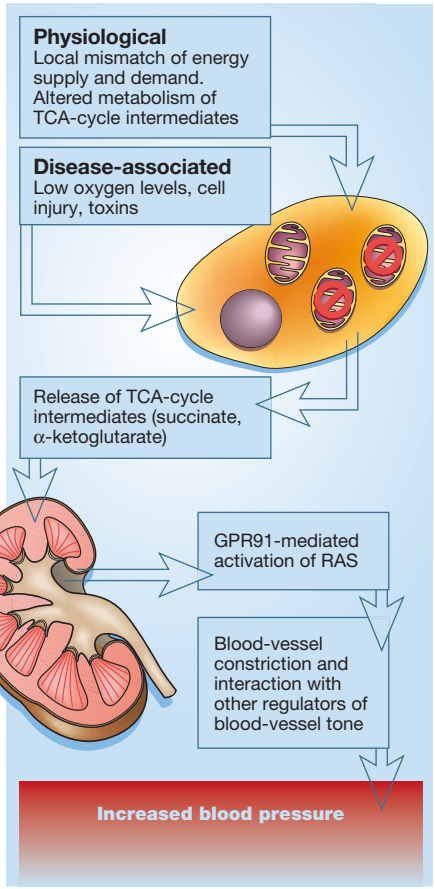


Figure 1 **Connecting metabolism to blood supply. He *et al.*[3] have found that the 'orphan' G-protein-coupled receptors GPR91 and GPR99 detect succinate and α-ketoglutarate — intermediates produced by the tricarboxylic acid (TCA) cycle during respiration. They also find that succinate increases blood pressure in mice. The figure shows how this happens. A local mismatch of energy supply and demand, altered metabolism of TCA-cycle intermediates, or injury leads to mitochondrial dysfunction and the release of succinate and α-ketoglutarate. These molecules activate receptors in the kidney, causing the release of renin and activation of the renin–angiotensin system (RAS). The RAS leads to an increase in blood pressure and altered local blood flow. Physiologically, this system might act to regulate local blood flow to match metabolic demands. However, it might also result in hypertension or alter cellular function.**

Muscle

# The sliding filament at 50

"In spite of the numerous investigations which have been made into the changes of the striations of muscle when it contracts, there is little agreement at the present day on either the nature or the significance of these changes." Thus started the first of two independent, ground-breaking papers[1,2], published together in *Nature* on 22 May 1954, which brought general agreement about those changes. The papers showed that muscle shortens by relative sliding between two sets of subcellular filaments containing the proteins myosin and actin. This was the first demonstration that a cell's primary function could be understood in terms of a fundamental interaction between two protein molecules.

In the first of the papers[1], Andrew Huxley and Rolf Niedergerke reported measurements of the optically dense 'A-bands' in intact fibres from striated (skeletal) muscle. Using a novel interference microscope, the authors demonstrated that the width of the A-bands remains constant during contraction. To account for their observations, they suggested a 'sliding-filament' model

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in which myosin filaments run the length of the A-band and actin filaments slide into this band when muscle shortens.

In the second paper[2], Hugh Huxley (no relation to Andrew) and Jean Hanson described their light-microscope investigations of isolated myofibrils from striated muscle; myofibrils are subfibres of muscle that are thinner and more suitable for light microscopy. Huxley and Hanson independently established the constancy of the A-band width and also invoked a sliding-filament model to explain their data. They also extracted myosin from the A-bands and demonstrated the role of ATP hydrolysis in powering the contraction–relaxation cycle of muscle.

Pictured here are the four protagonists. Clockwise from bottom left: Andrew Huxley, Rolf Niedergerke, Hugh Huxley and Jean Hanson.

The impact of the early work, and later developments in understanding the molecular mechanisms of what have since become known as motor proteins, are the subject of two meetings[3,4] to be held in London next week. The two classic papers are reproduced in full in a special web focus[5]. The web focus also includes selected *Nature* publications that subsequently advanced our understanding of the molecular basis of muscle contraction and its bearing on an intriguing issue — the biological conversion of chemical energy into mechanical work. **Maxine Clarke**


1. Huxley, A. F. & Niedergerke, R. *Nature* **173,** 971–973 (1954).
2. Huxley, H. E. & Hanson, J. *Nature* **173,** 973–976 (1954).
3. www.royalsoc.ac.uk/events/discussion_meetings/level_2/may04.html
4. www.kcl.ac.uk/175/events/event0520.html
5. www.nature.com/nature/focus/slidingfilaments


blood pressure (hypertension) through the activation of the RAS. A similar mechanism could perhaps account for, or contribute significantly to, the renin-mediated hypertension that is associated with constriction of the renal artery by cholesterol plaques, as occurs in diabetes, kidney disease and other conditions. If the TCA receptors are indeed involved, however, then receptor blockers might be expected to inhibit kidney renin secretion and relieve hypertension.

And there are further possible medical implications. The concentrations of TCA-cycle intermediates depend to a certain extent on the state of the enzymes that are responsible for their turnover. Succinate levels, for instance, might be increased when the mitochondrial activity of the complex containing succinate dehydrogenase and its activator, coenzyme $Q_{10}$ ($CoQ_{10}$), is low. Low concentrations of $CoQ_{10}$ occur after heart failure and hypertension. Oral administration of this coenzyme improves heart function in some patients[10], and some patients with hypertension can discontinue antihypertensive medications after taking $CoQ_{10}$ (ref. 11). It would be interesting to know whether these effects are linked to a decrease in extracellular concentrations of succinate and hence in the activation of the RAS. Another observation is that some drugs use succinate as a salt; could this be having an effect in some patients?

TCA-cycle intermediates such as α-ketoglutarate and succinate have also been shown to reduce the mitochondrial injury, in kidneys and other tissues, that is caused by successive decreases and increases in oxygen concentrations[12]; such intermediates are also used in kidney (and liver) preservation solutions for organ transplantation[13]. What role, if any, might the TCA receptors have here? And might these receptors function in tissues other than kidneys? The complete RAS is expressed in many tissues, including the placenta, brain, heart, gonads and pancreas[14,15]. Intriguingly, the placenta also expresses GPR99 (ref. 7), and disturbances in placental RAS activity can reduce placental blood flow in pregnancy, often with severe complications[14].

Many aspects of the function of the newly identified TCA receptors clearly remain to be determined. Similar exciting discoveries are likely to come as more and more orphan receptors find their homes in the GPCR family. ■


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