

NEUROSCIENCE

Controlled capillaries

Brian A. MacVicar and Michael W. Salter

The finest scale of blood flow through the brain occurs in capillaries. Suspicions that capillary flow is regulated by cells that put the squeeze on these vessels are now borne out by detailed experiments.

The control of brain blood flow poses an intriguing 'plumbing' problem. On the one hand, high overall flows are required to maintain healthy brain function, because in humans the brain accounts for 20% of the body's energy consumption even though it forms only 5% of the total weight. On the other hand, there is a need to precisely regulate increases and decreases in flow to match the changing metabolic needs of specific brain regions. It has been known for more than 100 years¹ that the brain regulates its own blood supply. It can increase blood flow specifically to discrete regions to follow increases in neuronal activity. This principle has been exploited in functional magnetic resonance imaging and positron emission tomography, which have been extensively used to map the brain regions that are associated with different tasks².

However, the cellular mechanisms by which specific brain regions regulate blood flow, and therefore their own nutrient intake and waste removal, are not fully understood. In their paper on page 700 of this issue³, Peppiatt *et al.* implicate new players in regulating blood flow at the smallest level in the brain — cells known as pericytes.

The large blood vessels supplying the brain

are the carotid and vertebral arteries, which then branch to form the network of pial arteries covering the surface of the brain. In the cerebral cortex, the pial vessels branch into smaller arteries, which enter the brain tissue itself and are called the penetrating arterioles. These arterioles branch into secondary and tertiary arterioles, until they reach the smallest vessel supplying the brain tissue, the capillary, which is only wide enough for one red blood cell to pass through it at a time. The capillaries then feed into the venuoles and veins, which carry the blood away.

By virtue of the smooth muscle that surrounds them, arteries and arterioles can regulate blood flow. Various processes, including the release of mediators from the endothelial cells that line these vessels, cause contraction or relaxation of the smooth muscle cells, thereby decreasing or increasing the diameter of the artery or arteriole — *et voilà*, as we know from Poiseuille's law of fluid dynamics, blood flow can be controlled.

It had been suspected that blood flow through capillaries is also regulated, even though there are no surrounding smooth muscle cells to constrict them. The suspicions were based on the observation that capillaries

are wrapped at intervals by a little-studied cell type with contractile capabilities, the pericyte. Peppiatt *et al.*³ now show definitively that pericytes in living tissue of the central nervous system can constrict and relax, correspondingly changing capillary diameter, and that they do this in response to changes in neuronal activity. Pericytes were identified by staining with a specific marker. The cells are spaced at intervals along capillaries, and also occur at capillary junctions. Their cell bodies abut the capillaries, with their processes wrapping them (see Fig. 1a of the paper³ on page 701).

Peppiatt *et al.* obtained the proof that pericytes modify capillary diameter by stimulating the cells directly with microelectrodes in rat brain slices and in retinal explants. They also found that drugs that activate purinergic receptors, a type of receptor found in the surface membrane of pericytes, stimulated pericyte-induced contractions. The constriction of the capillary occurred only in discrete regions or at capillary branch points. These types of localized change in capillary diameter should be very effective in blocking the entry of red blood cells, and thus slowing blood flow. The branch-point constriction might also divert flow into a capillary supplying an active region of neurons.

Inhibiting pericyte constrictions, by lowering the levels of external calcium (a common agent of cell signalling) or applying the neurotransmitter glutamate, caused dilation of pre-constricted capillaries, indicating that pericytes exert bidirectional control over capillary diameter. Significantly, the pericytes became constricted in response to stimulation by local neurons, showing that pericytes are sensitive to changes in neuronal activity.

DEVELOPMENTAL BIOLOGY

A change of heart

Vertebrate hearts have at least two chambers. But how did these evolve from the single-chambered pumps seen in simpler organisms? While examining the development of the heart of the sea squirt, Brad Davidson and colleagues (*Genes Dev.* **20**, 2728–2738; 2006) may have chanced on the answer.

The adult sea squirt (*Ciona intestinalis*, pictured) is a classic squishy invertebrate, but as a larval tadpole it resembles a fish embryo. This puts it closer to humans on the evolutionary scale than other genetic model organisms such as fruitflies and worms, and makes it a useful system for studying certain developmental processes.

Davidson *et al.* followed the single-chambered sea-squirt heart as it

grew from two cells in the early embryo. These cells divide and specialize to form muscle cells in either the heart or the tadpole tail. The authors find that the decision about which muscle type develops centres on a gene-regulatory factor called *Ets1/2* — cells in which the factor is active become heart cells.

But *Ets1/2* is also present, though inactive, in the cells destined to become tail. So something must activate it to set embryonic cells on the path to becoming heart cells. Davidson *et al.* teased apart the genetics to discover that the activating signal comes from a classic growth factor called FGF. Both *Ets1/2* and the FGF signalling pathway have relatives in vertebrates, and these have been



K. TELNES/IMAGE QUEST/MARINE

linked to heart development. This implies that the developmental pathway in the sea squirt has been conserved through evolution.

The twist in the tale came from an experiment in which Davidson and colleagues looked at the effect of a permanently active form of *Ets1/2* on embryonic cells destined to become tail. Not only did these cells develop into heart cells, confirming the role of *Ets1/2*, but in some animals the

resulting organ had two chambers rather than one.

So the authors speculate that since it separated from the sea-squirt lineage, an ancestral vertebrate recruited additional heart precursor cells to make a two-chambered heart. As their study shows, even a subtle change in signalling in the pool of cells that can form muscle cells might have allowed this transition.

Helen Dell

The constrictions could propagate from one pericyte to another along the capillary, which could provide a means by which small regions of the vascular system detect and respond to the activity of nearby groups of neurons.

The true impact of pericyte activity in the intact central nervous system still needs to be established. But modifying blood flow through the capillary 'bed' is likely to have a profound impact on overall blood flow. There is *in vivo* evidence from two-photon laser scanning microscopy that capillary blood flow can be dynamically controlled, and even reversed, in some cases^{4,5}. Are these changes at the capillary level due to the actions of pericytes, or do they result from upstream alteration of blood flow in the arterioles? This issue is complicated by the effects of neuronal inputs on blood flow in arteries and arterioles^{6,7}, or of cells called astrocytes, which can also constrict or dilate vessels⁸⁻¹⁰. Furthermore, localized arteriole constrictions have been observed following transient increases in intracellular calcium in astrocytes, but these were due to contractions of the smooth muscle cells, not to pericyte constriction^{8,9}. A future issue is to find out whether pericytes can also induce constrictions of small arterioles.

Ultimately, the goal is to understand the hierarchy of cerebral blood-flow control by the different elements that can alter vascular dilation and constriction. We will then know which levels are indeed important in inducing the changes in flow that underlie functional

imaging techniques. This knowledge is crucial for understanding the link between regional brain activation and blood flow, and for designing therapies for repairing this relationship in brain diseases such as vascular dementia. The known actors in controlling blood flow are endothelial cells, smooth muscle cells, neuronal inputs and astrocytes. Now, thanks to Peppiatt *et al.*, pericytes can be added to the cast. ■

Brian A. MacVicar is in the Brain Research Center, University of British Columbia, Vancouver, British Columbia V6T 2B5, Canada.

e-mail: bmacvica@interchange.ubc.ca
Michael W. Salter is in the Program in Neurosciences and Mental Health, Hospital for Sick Children, Toronto, Ontario M5G 1X8, Canada.
e-mail: mike.salter@utoronto.ca

- Roy, C. S. & Sherrington, C. S. *J. Physiol. (Lond.)* **11**, 85-108 (1890).
- Lauritzen, M. & Gold, L. *J. Neurosci.* **23**, 3972-3980 (2003).
- Peppiatt, C. M., Howarth, C., Mobbs, P. & Attwell, D. *Nature* **443**, 700-704 (2006).
- Chaigneau, E., Oheim, M., Audinat, E. & Charpak, S. *Proc. Natl Acad. Sci. USA* **100**, 13081-13086 (2003).
- Kleinfeld, D., Mitra, P. P., Helmchen, F. & Denk, W. *Proc. Natl Acad. Sci. USA* **95**, 15741-15746 (1998).
- Edvinsson, L. & Hamel, E. in *Cerebral Blood Flow and Metabolism* (eds Edvinsson, L. & Krause, D. N.) 43-70 (Lippincott Williams & Wilkins, Philadelphia, 2002).
- Cauli, B. *et al. J. Neurosci.* **24**, 8940-8949 (2004).
- Mulligan, S. J. & MacVicar, B. A. *Nature* **431**, 195-199 (2004).
- Metea, M. R. & Newman, E. A. *J. Neurosci.* **26**, 2862-2870 (2006).
- Zonta, M. *et al. Nature Neurosci.* **6**, 43-50 (2003).

is preferentially removed from the system. But apart from a transient spike of ¹³C-enriched rocks following the GOE (the cause of which is still being debated⁷), carbonate rocks that formed before and after 2.4 billion years ago show the same isotopic signature. Why didn't things change when oxygen levels went up?

According to Goldblatt and colleagues⁵, once photosynthesis began, the atmosphere became bistable: it could exist in either a low- or a high-oxygen state. This bistability results from variations in the rate of atmospheric oxygen consumption as oxygen levels change. In a low-oxygen atmosphere, oxygen is rapidly consumed in an ultraviolet-catalysed reaction with biogenic methane. But as oxygen levels increase, so does the concentration of ozone, which shields the atmosphere from solar ultraviolet radiation and thus abates oxygen consumption. Therefore, the atmospheric oxygen budget can change even if other sources and sinks of oxygen remain constant. So maybe the atmosphere got stuck in a low-oxygen state for a long time following the start of photosynthesis, even though oxygen levels were poised to go much higher.

To explain why it is curious that the carbon-isotope signature of rocks did not change after the GOE, one must consider how the long-term oxygen cycle works. Photosynthesis by bacteria (and later by algae and plants) produces oxygen, but it is the burial of these organisms in marine sediments that leaves excess oxygen behind in the atmosphere — this excess oxygen would otherwise be used up as the organisms decay. The organic carbon found in living organisms is depleted in ¹³C relative to ¹²C, so increases in the rate of organic-carbon burial should cause changes in the ratio of carbon isotopes dissolved in the ocean. The isotopic signature of carbonate rocks formed from these dissolved substances should mirror the isotopic changes in sea water.

But carbonate rocks are isotopically similar before and after the GOE (apart from the unexplained spike). In other words, the source of atmospheric oxygen — organic-carbon burial — seems to have remained constant with time, even though atmospheric oxygen levels have changed enormously. This led various authors⁸⁻¹⁰ to propose that the GOE was caused by decreases in the sinks for oxygen — that is, by lower emissions of reduced gases from beneath Earth's crust and by lower discharge rates of dissolved ferrous iron from hydrothermal vents. But all such proposals either have internal inconsistencies or violate other constraints provided by the geological record⁵.

Goldblatt *et al.* now suggest that a mere 3% increase in organic-carbon burial would have been enough to trigger the GOE. Such a change is far too small to be detected in the carbon-isotope record. A decrease in reductant input by the same tiny amount could also have prompted the GOE. Such minor fluctuations could have happened for any number of reasons. However, the authors show that a much larger perturbation is required to cause

EARTH SCIENCES

Ups and downs of ancient oxygen

James F. Kasting

The latest models suggest that atmospheric oxygen could have fluctuated between high and low concentrations once photosynthesis had evolved. But does the geological evidence really support this?

The ancient rise of atmospheric oxygen is of great interest because of its close relationship with evolution, but the geological evidence for this is indirect and subject to interpretation. The consensus for more than 30 years has been that atmospheric oxygen first reached appreciable levels around 2 billion to 2.4 billion years ago^{1,2}, an occasion known as the great oxidation event (GOE). But doubters of this event have remained³.

The GOE story was strengthened considerably by the discovery that minerals in ancient rocks had unusual ratios of sulphur isotopes, a phenomenon known as mass-independent fractionation⁴ (MIF). The only known mechanism that can produce this effect is the break-up of sulphur dioxide by ultraviolet light in a low-oxygen atmosphere. The MIF isotopic signature is small or entirely absent in rocks younger than 2.4 billion years, suggesting that

Earth's atmosphere has been oxygen-rich since that time⁴. In this issue, Goldblatt *et al.* (page 683)⁵ extend the oxygen evolution story, and in doing so may have found some common ground for GOE believers and heretics.

Goldblatt and colleagues⁵ do not challenge the conventional wisdom regarding the rise of oxygen. Instead, they present a model that might resolve two problems that have puzzled geochemists for years. First, why did atmospheric oxygen climb to significant levels only around 2.4 billion years ago, when oxygen-producing bacteria apparently evolved 2.7 billion years ago⁶, or earlier? Second, carbonate rocks that form on the sea floor should acquire a distinctive ratio of carbon isotopes, as organic carbon from photosynthesizing organisms gets buried in marine sediments. Increased organic-carbon burial should cause the ratio of ¹³C to ¹²C in carbonates to rise, because ¹²C