Professor Dan Berkowitz describes the initial rediscovery of the phenomenon of photorelaxation, whereby the muscular walls of blood vessels relax in response to light stimulation, as “a serendipitous finding.” He tells how a postdoctoral fellow in his lab noticed the phenomenon following a change to motion controlled lighting in the building they occupy. “Dr Sikka noticed that every time he walked into the laboratory and activated the lights, the blood vessels in his organ bath experiments relaxed,” he said. “After some further light-based experiments, we were convinced that light-induced relaxation occurs in blood vessels and we were excited to explore the phenomenon and its underlying mechanism.”

It wasn’t all plain sailing though, a search of the literature uncovered earlier work by the Nobel prize-winning vascular biologist Robert F. Furchgott. The team were initially disappointed when they discovered that the phenomenon had been described over 40 years earlier, but a closer look revealed significant gaps with the interpretation at the time. “The mechanisms thought to be responsible for this phenomenon were improbable and poorly understood,” said Prof Berkowitz. This sparked renewed interest from the team in getting to the bottom of this unusual activity.

**Heading to the light**

There are a limited number of ways that biological systems can detect and respond to light stimuli, the most commonly known of these being those related to sight. Opsins, or light receptors, are a group of evolutionarily ancient receptors which include visual opsin such as rhodopsin. These are present in the mammalian retina and are responsible for our ability to see. In contrast, non-visual opsins such as melanopsin are important in synchronising an organism to changes in light periods, the circadian rhythm. Prof Berkowitz hypothesised that it was these non-visual opsins such as melanopsin, which were responsible for the vasorelaxation they had witnessed in their laboratory setups. Designing a series of experiments to test this hypothesis, they set about bringing to light a hitherto misunderstood feature of vascular biology.

The first step was to discover and characterise which opsin receptors were present in the blood vessels which they studied. This was achieved by analyzing messenger RNA from vascular cells via the polymerase chain reaction (PCR) method. They found both melanopsin (Opn4) and panopsin (Opn3) in a variety of blood vessels. “Our finding that functional Opn4 receptors are present in the vasculature is consistent with nonvisual functions of Opn4,” said Prof Berkowitz, going on to point out that, “finding photoreceptors in blood vessels is not entirely surprising, because Opn4 is known to regulate retinal blood vessel development.”

**Putting the puzzle together**

So, the necessary photoreceptors are present, but are they responsible for the observed vasorelaxation and if so, what is the mechanism by which they act? The next piece of the puzzle lay in removing or blocking these receptors and running the experiments again. Using both specially bred mice which lacked the gene for Opn4 and chemical inhibitors of the receptors, the team showed that this abolished the photorelaxation effect observed in their experiments.

The way that vascular tone is measured experimentally is usually in a specialised apparatus called a myograph. Here two fine wires are inserted into the lumen of the blood vessel (the inside of the tube) and clipped to sensitive strain gauges. Slight tension is carefully applied to the blood vessel (the inside of the tube), and the team confirmed the maximal response. Having identified the receptor, further confirmation could be obtained by adjusting the wavelength of the light stimulus. Opn4 is known to be activated by short wavelength light (the blue end of the spectrum), and the team confirmed that light in this range produced the maximal response.

By first using light sources that just produced red, green or blue light, then later a monochromator which could adjust the wavelength in thirty-nanometer increments, they were able to narrow down the range of activity to 400-500 nm wavelength that constitutes what we perceive as blue light. The experiments showed the same effect as with the knockout mice. This clear evidence of Opn4 mediated photorelaxation of blood vessels was described by one commentator as, “A brilliant study… [which] provides an intriguing molecular explanation for a phenomenon that has puzzled vascular biologists for more than half a century.”

**Colour is key**

The team’s success didn’t stop there. Having identified the receptor, further confirmation could be obtained by adjusting the wavelength of the light stimulus. Opn4 is known to be activated by short wavelength light (the blue end of the spectrum), and the team confirmed that light in this range produced the maximal response. By first using light sources that just produced red, green or blue light, then later a monochromator which could adjust the wavelength in thirty-nanometer increments, they were able to narrow down the range of activity to 400-500 nm wavelength that constitutes what we perceive as blue light. The careful and progressive experimental design also took account of uncovering more of the mechanism by which the signal is transduced. They ruled out an endothelium (the cells lining the lumen) mediated pathway by removing the endothelium from the vessels before light stimulation. They further ruled out classical signalling pathways such as nitric oxide production using drugs which blocked production as well as chemical scavengers to mop up any remaining stores.

This list of ‘unsuccessful’ experiments was steadily leading them towards hyperpolarisation (a change in the voltage across a cell membrane due to movement of ions) of the vascular smooth muscle cells as an explanation for the effect. This was duly confirmed by electrophysiological recordings of smooth muscle cells in the preparation. This mechanism is related to that found in the ‘simple’ receptors of invertebrates, though the physiological mechanism...
Behind the Research

Professor Dan Berkowitz

A brilliant study... [which] provides an intriguing molecular explanation for a phenomenon that has puzzled vascular biologists for more than half a century.

The regulation of the dilation and constriction of blood is important as it regulates blood flow, a process that is abnormal in vascular disease. Berkowitz and his team have identified a new mechanism that regulates blood vessels; a type of light sensitive receptor that causes blood vessels to dilate when illuminated. They have thus identified a light-activated molecular switch that can be used to treat diseases such as Raynaud’s phenomenon and light sensitive diseases such as light induced pulmonary vasorelaxation that is potentiated by G protein-coupled receptor kinase 2 inhibition. ‘American Journal of Physiology, Vol. 314 (1). DOI: 10.1152/ajpheart.00917.2017’

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By inhibiting GRK2 and casting blue light to the periphery of the pulmonary vasculature in rats which exhibit pulmonary hypertension (high blood pressure in the pulmonary circulation) has widespread health effects, they were able to induce sustained vasorelaxation. This raises the potential of ‘phototherapy’ where GRK inhibitors and control of light exposure are used together to adjust vasoreactivity. Light stimulation, or other targeting of these open receptors, may provide another option for treatment in cases where current therapies are ineffective or undesirable.

What started as an energy saving measure (changing laboratory lighting controls), through being picked up by a keen-eyed researcher and developed by Prof Berkowitz and colleagues, has shed light on an area of vascular biology that has remained in the dark for nearly half a century. The implications of this research for the treatment of hypertension in the future could be significant, but here and now it is showing that with attention to detail, good experimental design, and just a little luck, there are still amazing discoveries to be made.

By which this hyperpolarisation comes about in the case of mammalian blood vessels, is complex and not easy to ascertain.

One interesting branch of the investigation looked at the rapidity with which the response is desensitised. This is clearly mediated by the G-protein receptor kinase GRK2, which phosphorylates the receptor (the addition of a phosphohydro group to a molecule, critical for regulating many biological processes) so that it cannot reactivate the signalling pathway. This fits with the nature of the Opn4 receptor and the team showed that the desensitisation was just as quick to recover, requiring a mere thirty minutes of darkness to return to normal response.

A BRIGHT FUTURE

Prof Berkowitz and his colleagues have gone on to show that this effect is more widespread than previously thought, with evidence from the pulmonary arteries (which carry blood from the heart to the lungs) of rats, cows and pigs. They have even begun to show the possibility of using this improved understanding of the action of Opn4 therapeutically.

The implications of this research for the treatment of hypertension in the future could be significant, but here and now it is showing that with attention to detail, good experimental design, and just a little luck, there are still amazing discoveries to be made.

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