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Robert F. Furchgott, Nobel Laureate (1916-2009) – A Personal Reflection

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Abbreviations: cGMP, cyclic guanosine 5´monophosphate; EDHF, endothelium-derived hyperpolarizing factor; EDRF, endothelium-derived relaxing factor; L-NMMA, N^G-monomethyl-L-arginine; NANC, non-adrenergic, non-cholinergic; NSAID, non-steroidal anti-inflammatory drug; PDE, phosphodiesterase; SOD, superoxide dismutase.

Summary

Robert F. Furchgott, pharmacologist and joint winner of the Nobel Prize for Medicine or Physiology (1998) died on the 12th of May 2009 aged 92. By unlocking the astonishingly diverse biological actions of nitric oxide, Furchgott leaves behind a rich legacy that has both revolutionised our understanding of human physiology and stimulated new and exciting opportunities for drug development in a wide range of pathological conditions. In this article, William Martin, who worked with Furchgott for two years (1983-1985) following the exciting discovery of endothelium-derived relaxing factor (EDRF)/nitric oxide, pays tribute to his close friend and colleague.

Introduction

May 12th 2009 saw the passing of Robert F. Furchgott (Bob to his friends and colleagues), perhaps the most accomplished and revered pharmacologist of his generation. There can be few in our discipline whose work has pervaded so many facets of biology and medicine and led to such a fundamental reappraisal of our understanding. Indeed, the awarding of the ultimate accolade, the Nobel Prize for Medicine or Physiology, to Furchgott (together with Ferid Murad and Louis Ignarro) in 1998, reflects the importance and world-wide impact of the discovery that nitric oxide plays a vital role in health and disease. There are many resources available to those seeking a comprehensive account of Furchgott's life and work as a scientist, but two of the most authoritative can be found on the Nobel website. The first is Furchgott's autobiography

(http://nobelprize.org/nobel_prizes/medicine/laureates/1998/furchgott-autobio.html)

in which he recounts his entry into science, his early work on receptor theory, including his introduction of the concept of receptor reserve and a method for

estimating the equilibrium dissociation constant for an agonist by using an irreversible antagonist. It also highlights his work on adrenergic mechanisms and the characterisation of receptors on blood vessels. In fact, these accomplishments alone would be regarded by most as a worthy life's work. He makes no mention here, however, of the discovery of endothelium-derived relaxing factor (EDRF)/nitric oxide – he reserves his personal account of this, the most celebrated of all his contributions, for the text of his Nobel lecture

(http://nobelprize.org/nobel_prizes/medicine/laureates/1998/furchgott-lecture.html). There is surely no more insightful a way to learn of the story of nitric oxide, the events and the personalities involved, than by reading a truly masterful account of it in the words of the great man himself.

Thus, Furchgott leaves behind so rich a legacy to his discipline that to do justice to it in a short tribute is an impossible task. Instead, I give here a personal reflection on the two super-charged years (1983-1985) I spent researching with him in his laboratory at the Downstate Medical Center in Brooklyn, New York. Although fully aware of just how privileged I was to get the opportunity to work with one of the world's greatest scientists who had just made the most important discovery of his career, the experience was to prove so much more. Getting to know Furchgott, a man of great sensitivity, humility, kind heartedness and warmth, who was destined to become a dear friend, added a most pleasant personal dimension to our interactions.

The discovery of EDRF

I first encountered Bob Furchgott in 1980 at Paul Vanhoutte's symposium on Mechanisms of Vasodilatation in Antwerp. As this was the year he published his now

legendary paper in Nature (Furchgott & Zawadzki, 1980), it is not difficult to imagine the raw excitement of this time. The electric atmosphere generated by Furchgott as he recounted his discovery of EDRF and the process of endothelium-dependent vasodilatation while the audience sat in stunned silence was surely one of the most inspiring moments in science. The importance of this was particularly acute to me, as I had just completed my PhD on non-adrenergic, non-cholinergic (NANC) inhibitory transmission with John Gillespie and had recently moved to postdoctoral position with John Gordon and Jeremy Pearson at the Babraham Institute in Cambridge, where they directed one of the few laboratories in the UK investigating vascular endothelial cell function.

Linking EDRF with NANC inhibitory transmission

As my own interest in endothelial cells developed and my postdoctoral funding drew to an end, I began communicating with Furchgott in the hope of securing a post in his lab. I drew to his attention the striking parallels between the work of John Gillespie's lab in Glasgow on the unidentified NANC inhibitory transmitter in the rat anococcygeus muscle (Gillespie, 1972) and his own work on EDRF. I explained that John Gillespie had assigned to me the task of trying to extract the NANC transmitter from the bovine retractor penis muscle (BRP), an anatomical extension of the anococcygeus, following publication of an abstract from Ambache's lab (Ambache *et al.,* 1975). Like Ambache, we succeed in extracting from the BRP a novel, labile smooth muscle relaxant that mimicked the action of its inhibitory nerves. We gave this the name "inhibitory factor", and went on to characterise some of its properties (Gillespie & Martin, 1980; Gillespie *et al.*, 1981). Its most unusual feature was that it was active if extracted in weak acid, but inactive if extracted at neutral pH; a brief

exposure of the latter to acid pH, optimum at pH 2, however, transformed it to its active form. These properties struck a chord with Furchgott, because it reminded him of an accidental finding he made in his own laboratory several years before, when sodium nitrite had been prepared in error in an acidic rather than a neutral solution and had transformed the weak long-lasting relaxant into a more potent ephemeral agent. As will be seen later, these observations contributed to his eventual conclusion that EDRF and the inhibitory factor were nitric oxide. The other major aspect that excited Furchgott was that the late Anne Bowman, who joined Gillespie's lab in the last year of my PhD studies, showed that inhibitory factor was a powerful vasodilator *in vitro* (Bowman *et al.*, 1981). Surprisingly however, inhibitory factor had absolutely no effect on the blood pressure of the anaesthetised rat. In her own characteristically tenacious manner, Anne strove to solve this puzzling anomaly. Convinced it was blood that was inactivating inhibitory factor, Anne invited me to join her in one last experiment before I left Glasgow. She harvested some whole heparinised blood, spun an aliquot to obtain erythrocyte and plasma fractions, and mixed each of them with BRP extract, which I then bioassayed. Anne's conviction had been correct, blood was indeed able to block the actions of inhibitory factor, and the agent responsible resided in the erythrocytes but not the plasma (Bowman *et al.*, 1981). Of course, later work by Anne, John Gillespie and David Pollock established that it was haemoglobin that inactivated inhibitory factor, and even more importantly, that it also blocked in a highly selective manner NANC inhibitory transmission in the BRP (Bowman *et al.*, 1982; Bowman & Gillespie, 1982). Thus, haemoglobin, which we now know interacts rapidly with nitric oxide, resulting in the formation of nitrate and methaemoglobin, became established as the first useful tool to modulate the activity of the NANC

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inhibitory nerves. Inspired by these parallels to his own work on EDRF, Furchgott required no further persuasion to allow me to join his laboratory.

I arrived in New York at a particularly bleak time for Furchgott; his wife Lenore was in a hospice and died some two weeks later. In spite of his difficulties at this time, Furchgott went out of his way to make me, my wife Anne and son Steve, feel very welcome and provided all sorts of practical help in getting settled in our new environment. Over the next few months we grew very close and informally adopted him into our family. Thursday became our relaxed beer and pizza night and we often had him over for a proper dinner at the weekend too. I would like to think that as our friendship grew that we helped him through those dark days following the loss of his wife. We shared so much both professionally and socially in those two terrific years. Bob introduced us to the delights of New York City, Long Island and to sailing and fishing in Long Island Sound. In turn, we took him to our home in Glasgow in the summer of 1984 and toured the mountains, lochs and castles of the Scottish Highlands. Such were the beginnings of a long and deep personal friendship and professional association.

Haemoglobin inactivates EDRF

On my arrival in the Furchgott laboratory, it was obvious that we, together with our colleagues Gina Villani and Desingaro Jothianandan, should investigate whether haemoglobin had the same inhibitory effects on endothelium-dependent relaxation as it did on NANC inhibitory transmission. Indeed it did, and in a spectacularly selective manner that permitted the endothelium-independent relaxant action of isoprenaline to operate entirely unhindered (Martin *et al.*, 1985a; Martin *et al.*, 1985b). From that

point on our conviction grew that the identities of EDRF and the NANC inhibitory transmitter were linked. Indeed, a rapid series of events in different laboratories around the world confirmed this to be true. The next important lead came from Murad's lab which showed that endothelium-dependent relaxation of the rat aorta was associated with an elevation in smooth muscle cGMP content (Rapoport & Murad, 1983), through an action on soluble guanylate cyclase. This was the same pathway that Murad's group had shown some years earlier underpinned the relaxant actions of the nitrovasodilators, nitroprusside and glyceryl trinitrate, and even nitric oxide itself (Katsuki *et al.*, 1977). Thus, the effector pathway and second messenger system used by EDRF to promote smooth muscle relaxation was established. This was quickly followed by a paper from the Glasgow lab showing that relaxation of the BRP by the NANC inhibitory nerves or by inhibitory factor was also associated with a rise in cGMP content and, furthermore, that haemoglobin abolished all of these actions (Bowman & Drummond, 1984). In the Furchgott lab we demonstrated that haemoglobin also abolished the rise in cyclic GMP induced by EDRF in the rabbit aorta (Martin *et al.*, 1985b). Furthermore, as is often the case with the introduction of a new pharmacological tool, haemoglobin provided a host of other new and unexpected insights. For example, although it had no effect on the tone of rat or rabbit uncontracted aortic rings, in submaximally contracted tissues, haemoglobin augmented tone if the endothelium was present but not if it had been removed (Martin *et al.*, 1985b; Martin *et al.*, 1986b). Interestingly, when haemoglobin produced this enhancement of vasoconstrictor tone, it lowered the cGMP content of the aortic rings, consistent with the earlier observation in Murad's lab that the resting level of this cyclic nucleotide was lower in endothelium-denuded than in endothelium-containing rings (Rapoport & Murad, 1983). Thus, the use of haemoglobin in Furchgott's lab

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provided the first evidence that a basal (unstimulated) release of EDRF suppressed vasoconstrictor tone in the vasculature, and this was, of course, confirmed later upon the introduction of inhibitors of nitric oxide synthase (Rees *et al.*, 1989; Moore *et al.*, 1990; Rees *et al.*, 1990). The physiological and pathological implications of the ability of haemoglobin to inhibit EDRF were not lost on Furchgott; he concluded that EDRF must be a local autacoid and not a circulating hormone, and that haemoglobin might be responsible for the profound vasospasm associated with haemorrhage, in particular that following haemorrhagic stroke (Furchgott *et al.*, 1985a).

Shedding more light

In parallel with the work on haemoglobin on EDRF, Furchgott persuaded others in the lab, principally Desingaro Jothianandan, to pursue his other major discovery and great passion, the phenomenon of photorelaxation (by UV light). This he first observed when a cloud lifted and sunlight irradiated his tissue baths contain rabbit aortic strips (Furchgott *et al.*, 1961). Intriguingly, photorelaxation of the rabbit aorta did not require the presence of the endothelium, but as with relaxation induced by EDRF, it took place in association with an elevation of smooth muscle cGMP content, and these actions were virtually abolished by haemoglobin (Furchgott *et al.*, 1984; Furchgott *et al.*, 1985b). As a consequence, Furchgott became convinced that the "photo-activated relaxant factor" was linked in some way to EDRF and the inhibitory factor from BRP. We know now of course that photorelaxation proceeds through the photolytic decomposition of smooth muscle S-nitrosothiols and the resultant liberation of nitric oxide (Megson *et al.*, 2000; Ng *et al.*, 2007). Thus evolved yet another strand in the tangled web that led Furchgott ultimately to nitric oxide (Furchgott, 1988).

Information from phosphodiesterase (PDE) isoform 5 inhibition

In another development at that time, the Glasgow group showed that the selective PDE 5 inhibitor, M&B 22948 (zaprinast), produced a powerful enhancement of NANC inhibitory transmission and associated elevation of cGMP in the BRP muscle (Bowman & Drummond, 1984). Shortly thereafter, the Furchgott lab demonstrated similar findings in rat and rabbit aorta, whereby M&B 22948 and the non-selective PDE inhibitors, papaverine and isobutylmethylxanthine, produced endothelium-dependent relaxation, not through the stimulated release of EDRF, but by potentiation of basal EDRF activity resulting from the slowed hydrolysis of cGMP (Martin *et al.*, 1986a). This was the extent of Furchgott's involvement with PDE 5 inhibitors. Contrary to what has been portrayed in the popular media, Furchgott never worked on or was involved in the development of Pfizer's anti-impotence treatment, Viagra (sildenafil). It is therefore likely that certain sensationalist headlines in the media reporting his death, such as "US Viagra scientist dies at 92", "Viagra developer Furchgott dies at 92", have caused some distress to his family and close friends. Yes, he helped pave the way for its development, but had no involvement with Pfizer. It is perhaps a sad reflection on some of our major news agencies that they failed to report the true nature of his life's work and its importance.

The final piece of the puzzle

With the expiry of both my funding and visa, my involvement in the Furchgott lab ended and I returned to the UK with world events taking place at an ever increasing pace. Independent reports from the laboratories of Vanhoutte and Moncada demonstrated that superoxide anion rapidly destroyed EDRF, while superoxide

dismutase (SOD) potentiated and prolonged its actions (Rubanyi & Vanhoutte, 1986; Gryglewski *et al.*, 1986). This proved to be the final piece in the puzzle for Furchgott. He subsequently reported at the 1986 symposium on Mechanisms of Vasodilatation that superoxide anion and SOD had identical effects on the biological activities of EDRF and nitric oxide, the latter generated using acidified nitrite (Furchgott, 1988). More importantly, he reviewed the literature to-date and on the basis of similar physical, chemical and pharmacological properties concluded that both EDRF and the inhibitory factor extracted from the BRP muscle were nitric oxide. In fact, at the same meeting Ignarro also proposed that EDRF was nitric oxide on the basis of spectral data showing their identical interactions with haemoglobin (Ignarro *et al.*, 1988). It is somewhat unfortunate that a dispute with the publisher delayed the publication of these two reports, but with the major players in the field assembled at the symposium, the genie was out of the bottle – EDRF was looking increasingly like nitric oxide.

Confirmation that EDRF and the NANC inhibitory transmitter are nitric oxide

We largely have the Moncada group to thank for completing the story; using a cascade bioassay system linked to a chemiluminescence detector they proved not only that nitric oxide was indeed released from endothelial cells, but it was released in amounts that accounted fully for the biological actions of EDRF (Palmer *et al.*, 1987). In an accompanying Commentary in Nature, Vanhoutte announced "The end of the quest?" (Vanhoutte, 1987). Yes, this particular quest had indeed ended, but another had immediately begun – that to identify the precursor and synthetic pathway from which nitric oxide was derived. Soon thereafter the Moncada group revealed that nitric oxide was synthesised from a guanidino nitrogen of L-arginine (Palmer *et al.*, 1988) and that an N^G-substituted analogue, N^G-monomethyl-L-arginine (L-NMMA),

which we now recognise as an inhibitor of nitric oxide synthase, blocked in a competitive and stereo-specific manner, both the release of nitric oxide from rabbit aorta and the associated EDRF-mediated relaxation (Rees *et al.*, 1989). It was a satisfying end of the quest too for John Gillespie who had pursued the identity of the NANC inhibitory transmitter for so many years. He retired soon thereafter, content in the knowledge that he, simultaneously with two other groups, had identified the NANC transmitter too as nitric oxide (Li & Rand, 1989; Gillespie *et al.*, 1989; Ramagopal & Leighton, 1989). Thus, the L-arginine-nitric oxide pathway had become firmly established as a vital signalling system in biology.

The lasting legacy of Furchgott

Furchgott knew in 1980 when he first published on the phenomenon of endothelium-dependent vasodilatation that he was on to something big. Even he, however, could never have contemplated the scale of what he had started. The account above was merely the beginning, the field rapidly expanding thereafter into man, elucidating the astonishingly diverse roles played by nitric oxide in human physiology and disease. Those wishing to keep up with work that continues to develop from Furchgott's legacy may find the Journal's new "Virtual Endothelial Issue" to be a valuable resource (http://www.brjpharmacol.org/view/0/VirtualIssues.html). In addition to nitric oxide, topics highlighted here include, signalling by other endothelium-derived mediators such as endothelium-derived hyperpolarizing factor (EDHF) (Michel *et al.*, 2008; Garland & Dora, 2008), nitroxyl anion (Martin, 2009; Andrews *et al.*, 2009), endothelin (Farhat *et al.*, 2008; de Andrade *et al.*, 2009) and prostanoid contractile factors (Félétou *et al.*, 2009).

Furchgott's legacy extended yet wider still, sparking investigations into the functions of nitric oxide throughout the animal kingdom and in plants. From a pharmacologist's perspective, the nitric oxide saga has been an exceedingly fruitful area for therapeutics; it uncovered the mechanism of action of the nitrovasodilators that had hitherto been employed empirically for over 100 years to treat angina, and set the scene for the development of a vast array of new drug treatments. Present examples include nitric oxide itself for respiratory failure in premature infants and in adult respiratory distress syndrome, and PDE 5 inhibitors for the treatment of impotence and, more recently, pulmonary hypertension. Future promise is widely anticipated in a number of areas, including the development of selective inhibitors of inducible nitric oxide synthase for septic shock and a host of other inflammatory and immune diseases, selective inhibitors of neuronal nitric oxide synthase for the treatment of stroke and other neurological pathologies, and nitric oxide-releasing non-steroidal anti-inflammatory drugs to limit the gastric bleeding and ulceration commonly encountered with NSAIDs. In short, Furchgott's legacy shows no limits and looks set to expand for decades to come. He was a brilliant scientist and greatly admired character who changed the world – but fortunately the world did not change him – he remained ever humble in the face of growing fame and celebrity. The world mourns the loss of one of the great gentlemen of science.

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