Bioassay of prostacyclin and endothelium-derived relaxing factor (EDRF) from porcine aortic endothelial cells

R.J. Gryglewski, S. Moncada & R.M.J. Palmer

Commentary by

S. Moncada

This paper represented the interface between two long and very productive periods of research. The first of these started in 1976 with our discovery of thromboxane synthase (Needleman *et al.*, 1976) and prostacyclin (Moncada *et al* 1976a) and the second began with this paper and led to the discovery the following year of the identity of EDRF as nitric oxide (NO) (Palmer *et al.*, 1987) and the subsequent elucidation of its many biological roles.

Endothelium-dependent relaxation and EDRF had been identified by Furchgott and Zawadzki (1980) using fresh vascular tissues and many people had confirmed their findings using a variety of tissues (see Furchgott, 1984; Moncada *et al.*, 1987); Vanhoutte, 1989). A number of experiments were carried out attempting to transfer biologically active EDRF from one piece of vascular tissue to another. Some of these were sufficiently successful for a half-life for EDRF to be calculated (Griffith *et al.*, 1984; Cocks *et al.*, 1985; Forstermann *et al.*, 1985). Many other experiments were carried out attempting to identify the chemical structure of EDRF and to inhibit its activity (Singler *et al.*, 1984; Pinto *et al.*, 1985; MacDonald *et al.*, 1986).

We tried to approach the problem in a different way. The difficulties associated with the research on EDRF at that time were related to the indirect nature of the majority of the experiments in which endothelium-dependent relaxation was measured or, when working with endothelium-derived relaxing factor, to the very small amounts of material that were being used. We thought that if proper pharmacological studies were to be carried out and the chemical structure was to be identified then we needed a larger quantity of material and a reliable and, as much as possible, a quantitative bioassay system. We decided to culture endothelial cells on microcarrier beads and to perfuse them in a modified chromatography column. We used the perfusate to superfuse a series of bioassay tissues, both vascular and non-vascular, which when combined appropriately allowed the differential bioassay of prostacyclin and EDRF.

The system was useful in several ways since, first, it allowed the culture of many millions of endothelial cells which opened the door to further important studies. Secondly, although the cells were not as responsive as vascular tissue, they did respond to substances such as bradykinin and the Ca^{2+} ionophore A23187 by releasing both prostacyclin and EDRF and this allowed the study of some of the biological characteristics of EDRF itself. Thirdly, the bioassay superfusion technique, as many times before (see Vane, 1964), proved to be immensely informative and reliable.

We were particularly well placed to do this work since, during the mid-1970s, we had gained a great deal of experience doing bioassay of unstable arachidonic acid metabolites such as prostacyclin and thromboxane A_2 (TXA_2), which had a half life of approximately 30 s. With Stuart Bunting we had struggled and succeeded in bioassaying TXA_2 generated from different sources (Moncada *et al.*, 1976b) and that experience was extremely useful in the study of EDRF which has a half-life of 4 to 7 s on the bioassay cascade!

Within a few months of our development of this method for the bioassay of EDRF, the technique allowed us to demonstrate that superoxide anion (O_2^-) inactivated EDRF (Gryglewski *et al.*, 1986) and this led to the elucidation of the mode of action of many inhibitors of EDRF as generators of O_2^- (Moncada *et al.*, 1986). These two papers provided important clues for the later identification of EDRF as NO.

The identification of NO (Palmer *et al.*, 1987; Khan & Furchgott, 1987; Ignarro *et al.*, 1987) and later discovery of its metabolic route from L-argi-

nine (Palmer *et al* 1988) thus identified a biochemical pathway which suddenly brought together many fields of research which until that time had had no apparent connection. These included studies on endothelium-dependent relaxation, on activators such as L-arginine of the soluble guanylate cyclase in the brain, on the release of nitrite and nitrate by activated macrophages and on non-adrenergic non-cholinergic neurotransmission (see Moncada & Higgs, 1990).

Looking back now, it is remarkable to what extent bioassay was the foundation of this field of research. As we now see molecular biology adding the final touches to this discovery and looking at its significance from a "gene point of view", we can safely say that pharmacology in its most classical manifestation, bioassay, is thriving and is as productive as ever!

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