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Hypothesis

Endothelium-derived relaxing factor is a nitrosyl iron complex with thiol ligands

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A hypothesis is put forward on the nature of the endothelium-derived relaxing factor (EDRF) which is released from vascular endothelial cells by acetylcholine, bradykinin and other agonists. It is suggested that EDRF is a nitrosyl iron complex with low-molecular thiol ligands, most probably with cysteine. Its active principle is nitric oxide (NO). This free radical is stabilized by inclusion into the iron complex, which promotes NO transfer within the cell and between cells. Subsequent release of NO from these complexes results from thiol group oxidation.

Endothelium-derived relaxing factor; Nitric oxide; Nitrosyl non-heme iron complex; Free iron

1. INTRODUCTION

Furchgott and Zawadsky discovered in 1980 that acetylcholine, among other substances, stimulates the vascular endothelium to release a factor capable of relaxing blood vessels, named endothelium-derived relaxing factor (EDRF) [1]. Its nature remains unclear. Most researchers suggest that the active principle of EDRF is nitric oxide (NO) [2–12]. Both EDRF and NO relax the vascular smooth muscle through the stimulation of soluble guanylate cyclase [13,14]. They share similar half-lifes in media containing superoxide radicals [3,4,14]. When reacting with O_2^- radicals, both EDRF and NO are transformed to nitrite and nitrate anions [15,16].

Despite these similarities, EDRF and NO are not identical. EDRF is more potent and long-lasting as a vasodilator [10,12]. Unlike NO, EDRF is not able to produce paramagnetic nitrosyl complexes with heme and non-heme iron [17,18]; it can not be evaporated from an aqueous solution [12,19]; it is trapped by some resins [20]. Finally, there is evidence that EDRF does not relax non-vascular smooth muscles, unlike NO which easily induces their relaxation [21,22].

Abbreviations: DNIC, dinitrosyl iron complex; DETC, diecthyldithiocarbamate anion; EDRF, endothelium-derived relaxing factor; ESR, electron spin resonance; MNIC, mononitrosyl iron complex; NO, nitric oxide

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For these reasons most investigators suggest that EDRF is a labile nitroso compound capable of releasing nitric oxide when interacting with vascular smooth muscle cells [3,8,10,12,22–24]. It is suggested that NO included in this compound is protected from various intracellular reagents, thus increasing its cellular life-time. At present, the most popular candidate for EDRF is an *S*-nitrosothiol, with the NO counterpart being most probably the thiol ligands, cysteine or glutathione [23,24]. Some data show similar physico-chemical characteristics and vasodilatory activities between EDRF and *S*-nitrosothiols [10]. Nevertheless, this idea seems unrealistic. Its weak point is the very low stability of nitrosothiols in aqueous medium within the neutral pH-range. The nitrosothiols are stable in acid solutions only. Thus it is necessary to speculate that they are formed and stored in special vacuoles with a highly acid inner phase [24]. This condition seems to be quite improbable and artificial.

2. PROPOSAL

This instability may be overcome by proposing that EDRF contains not only NO and thiols, but also iron, i.e. EDRF is a nitrosyl–non-heme-iron complex with thiols. These complexes were found in cells and animal tissues a long time ago [25–31]. Such complexes are sufficiently stable in neutral solution. Their structure and production conditions have been thoroughly studied [32–35]. They are dinitrosyl iron complexes (DNIC) with protein paired thiol groups or similar low-molecular compounds. These species are paramagnetic, their electron spin $S = 1/2$, an unpaired electron being

localized mainly at the iron atom with electron configuration d^7 (informal valence state Fe(I)). The nitrosyl groups exist in these complexes as nitrosonium-cations NO^+ [32]. They give an electronspin resonance (ESR) signal with axial anisotropy of g-tensor $g_\perp = 2.037$, $g_\parallel = 2.012$, $g_{av} \approx 2.03$ [32] and are usually named '2.03 complexes', according to their $g_{av.}$ value.

3. WHAT ARE THE FACTS AND EVIDENCE FOR THIS HYPOTHESIS?

1. The existence of 2.03 complexes in animal cells and tissues, when treated with nitric oxide in vitro and in vivo, as mentioned above. Recently, it was shown that 2.03 complexes occur also in activated macrophages which are capable of producing nitric oxide from L-arginine [36,37], the latter being the precursor of EDRF [5,6].

2. The presence in cells and tissues of two forms of 2.03 complexes, i.e. those bound to protein through their RS^--groups, and those containing low-molecular thiol ligands, cysteine or reduced glutathione [38]. Protein 2.03 complexes are more stable, with the equilibrium distribution of $Fe(NO)_2$-groups between low-molecular and protein complexes strongly shifted towards the latter [38]. Nevertheless, the inclusion of small quantities of $Fe(NO)_2$-groups into low-molecular 2.03 complexes provides their transfer within and between the cells. The existence of such a transfer is shown by the behaviour of protein 2.03 complexes after their injection into the blood of rats in vivo. After 10–20 min, the same complexes are found not only in the blood, but also in various tissues of these animals [39]. In model experiments on $Fe(NO)_2$-transfer between two protein solutions separated by an artificial membrane, it was shown that such a transfer occurs in the presence of low-molecular species capable of binding $Fe(NO)_2$-groups. These findings suggest that DNIC with small ligands may be identical to EDRF, released from endothelial cells and acting on neighbouring smooth muscle cells. Their thiol groups can be easily oxidized, which releases iron and nitric oxide.

At lower cysteine levels the DNIC with this ligand form dimeric and higher structures in aqueous solutions, similar to the red and black Roussin's salts [40,41]. This, if EDRF is the nitrosyl iron complex with cysteine, it can be in a dimeric (or polymeric) form.

3. It is noteworthy that cysteine is released from activated endothelial cells producing EDRF [42], and that cells release iron when treated with nitric oxide [43,44]. I predict that iron will be released from the cells producing EDRF or nitric oxide as well.

It is suggested that iron released from the NO-treated cells is derived from active sites of FeS-proteins [36,37,43–46]. In my opinion, the main source of iron is the loosely bound non-heme iron, the so-called free iron [47]. This iron forms 2.03 complexes [47–49] and it is released from the cells in these low-molecular species.

4. 2.03 complexes display hypotensive properties, they act as vasodilators in isolated blood vessels and inhibit platelet aggregation [39, 50–52]. These activities are due to nitric oxide. The two-phase kinetics of their effect has been found in animals, both narcotized or conscious. After a single dose of low-molecular 2.03 complexes, a fast initial decrease of arterial pressure is followed by a long-lasting (several hours) and steady hypotension in narcotized animals, or by a slow recovery (during 1.5–2 hours) in conscious animals. During the steady-state phase the main fraction of $Fe(NO)_2$ groups has been transferred from low-molecular to protein thiol groups. The formed protein 2.03 complexes provide a long-lasting hypotensive effect by gradually releasing nitric oxide, i.e. they act as a molecular storage of NO. The fast initial decrease in arterial pressure may be due to NO release from the small low-molecular fraction of DNIC which did not transfer their $Fe(NO)_2$ groups to protein.

Similar fast hypotensive effects may be achieved by protein 2.03 complexes when treated in the organism by diethyldithiocarbamate (DETC) [39]. With Fe^{2+} and NO this anion produces the paramagnetic mononitrosyl iron complexes (MNIC). These complexes are much more stable than 2.03 complexes. DETC removes from each of them a single iron ion and a single NO molecule, producing MNIC [53]. The unconsumed NO is then released in free form and induces a strong but transient hypotensive effect.

The above mentioned suggestion concerning the nature of EDRF is a working hypothesis requiring detailed experimental testing. First, the vasodilating properties of EDRF and 2.03 complexes should be compared; second, using the ESR technique, we need to verify whether EDRF and 2.03 complexes are really identical. Until recently, no data have been obtained on EDRF paramagnetism [18,24], probably due to the low concentration of EDRF in solution — about 10^{-8} M [10].

We showed recently [54] that NO-synthase in the cytosol from endothelial cells produces directly free NO. Apparently, the same process takes place in intact cells. Free nitric oxide would then bind to iron and thiol ligands forming EDRF. This is a way to stabilize NO in cells, and to provide both NO-transfer in tissues and its release in the active free state.

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