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Review

Prostacyclin among prostanoids

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Abstract:
Prostanoids are cyclic lipid mediators which arise from enzymic cyclooxygenation of linear polyunsaturated fatty acids, e.g. arachidonic acid (20:4 n 6, AA). Biologically active prostanoids deriving from AA include stable prostaglandins (PGs), e.g. PGE_2, $PGF_{2\alpha}$, PGD_2, PGJ_2 as well as labile prostanoids, i.e. PG endoperoxides (PGG_2, PGH_2), thromboxane A_2 (TXA_2) and prostacyclin (PGI_2). A "Rabbit aorta Contracting Substance" (RCS) played important role in discovering of labile PGs. RCS was discovered in the Vane's Cascade as a labile product released along with PGs from the activated lung or spleen. RCS was identified as a mixture of PG endoperoxides and thromboxane A_2. Stable PGs regulate the cell cycle, smooth muscle tone and various secretory functions; they also modulate inflammatory and immune reactions. PG endoperoxides are intermediates in biosynthesis of all prostanoids. Thromboxane A_2 (TXA_2) is the most labile prostanoid (with a half life of 30 s at 37°C). It is generated mainly by blood platelets. TXA_2 is endowed with powerful vasoconstrictor, cytotoxic and thrombogenic properties. Again the Vane's Cascade was behind the discovery of prostacyclin (PGI_2) with a half life of 4 min at 37°C. It is produced by the vascular wall (predominantly by the endothelium) and it acts as a physiological antagonist of TXA_2. Moreover, prostacyclin *per se* is a powerful cytoprotective agent that exerts its action through activation of adenylate cyclase, followed by an intracellular accumulation of cyclic-AMP in various types of cells. In that respect PGI_2 collaborates with the system consisting of NO synthase (eNOS)/nitric oxide free radical (NO)/guanylate cyclase/cyclic-GMP. Both cyclic nucleotides (c-AMP and c-GMP) act in synergy as two energetic fists which defend the cellular machinery from being destroyed by endogenous or exogenous aggressors. Recently, a new partner has been recognized in this endogenous defensive squadron, i.e. a system consisting of heme oxygenase (HO-1)/carbon monoxide (CO)/biliverdin/biliverdin reductase/bilirubin.
The expanding knowledge on the pharmacological steering of this enzymic triad (PGI_2-S/eNOS/HO-1) is likely to contribute to the rational therapy of many systemic diseases such as atherosclerosis, diabetes mellitus, arterial hypertension or Alzheimer disease. The discovery of prostacyclin broadened our pathophysiological horizon, and by itself opened new therapeutic possibilities. Prostacyclin sodium salt and its synthetic stable analogues (iloprost, beraprost, treprostinil, epoprostenol, cicaprost) are useful drugs for the treatment of the advanced critical limb ischemia, e.g. in the course of Buerger's disease, and also for the treatment of pulmonary artery hypertension (PAH). In this last case a synergism between prostacyclin analogues and sildenafil (a selective phosphodiesterase 5 inhibitor) or bosentan (an endothelin ET-1 receptor antagonist) points out to complex mechanisms controlling pulmonary circulation. At the Jagiellonian University we have demonstrated that several well recognised cardiovascular drugs, e.g. ACE inhibitors (ACE-I), statins, some of β-adrenergic receptor antagonists, e.g. carvedilol or nebivolol, anti-platelet thienopyridines (ticlopidine, clopidogrel) and a metabolite of vitamin PP – N^1-methyl-nicotinamide – all of them are endowed with the *in vivo* PGI_2-releasing properties. In this way, the foundations for the Endothelial Pharmacology were laid.

Key words:
Vane Bioassay Cascade, RCS (Rabbit aorta Contacting Substance), prostacyclin, thromboxane, prostaglandins, prostaglandin endoperoxides, arachidonic acid, eicosanoids, critical limb ischemia, pulmonary artery hypertension, atherosclerosis, diabetes mellitus, angiopathies, the endothelial pharmacology

Introduction

Prostanoids (PGs, PGI_2, TXA_2) belong to the superfamily of eicosanoids [in Greek eicosa (ειχοσα) stands for twenty – in that case twenty carbon atoms in a molecule]. Indeed, prostanoids derive from eicosa-all cis-5,8,11,14-tetraenoic acid, i.e arachidonic acid (AA). AA may be subdued to a number of enzymic manipulations [8]. Firstly, phospholipase A_2 (PLA_2) cuts it out from the cellular phospholipid stores. Next, free AA is exposed to the enzymes available in various types of cells and in various cellular compartments. E.g. cytosolic 5-lipoxygenase (5-LOX) opens the gate for cytotoxic leukotriens. In contrast with 5-LOX, the microsomal cytochrome P450-dependent epoxygenase system produces cytoprotective epoxyeicosatrienoic acids (e.g. 5,6- or 11,12-EETs). For us the most interesting are PGH synthases, better known as cyclooxygenases 1 and 2 (constitutive COX-1 and inducible COX-2). These generate prostaglandin endoperoxides (PGG_2 and PGH_2). PGH_2 is the substrate for various microsomal synthases, including thromboxane synthase (TX-S) and prostacyclin synthase (PGI-S) [8]. In blood platelets COX-1 is the main supplier of PG endoperoxides from which thromboxane synthase (TXA-S) generates thrombogenic thromboxane A_2. Aspirin at low doses is a pretty selective inhibitor of COX-1 in blood platelets, hence aspirin is effective against myocardial infarction. However, in a special category of "aspirin-sensitive" patients aspirin itself and other non-steroidal anti-inflammatory drugs may precipitate asthmatic attacks, probably owing to the removal of PGE_2 or other bronchodilator PGs from the bronchial tree, and giving an upper hand to leukotrienes [53, 54]. COX-2 is to be found in inflamed tissues, but also in vascular endothelium which is exposed to the shear stress. In the endothelium COX-2 supplies PGI-S with PGH_2. It is why the selective COX-2 inhibitors (coxibs), effective as anti-inflammatory drugs, may also precipitate thrombotic episodes in patients (at least so it happened in the case of rofecoxib).

The early days of prostaglandins

Ulf Svante von Euler of Karolinska Institutet in Stockholm (the Nobel Prize Laureate with Bernard Katz and Julius Axelrod in 1970 for discovering the neurotransmitters in the nerve endings) was the first who in 1935 used the name *prostaglandin* for a lipid factor that he extracted from *glandula prostatae***,** and that factor contracted smooth muscle of various organs [8]. In 1982 Sune Bergström, Bengt Samuelsson and John Vane were awarded the Nobel Prize for their discoveries in the field of prostaglandins (Fig. 1).

In 1960 Sune Bergström and his coworkers isolated from the biological material cyclic lipids. Their chemical structures were established, and they received the names of prostaglandins (PGs), precisely PGE_2 and $PGF_{2\alpha}$ (denomination E or F stayed for a better solubility in ethyl ether or in phosphate buffer, and the index "2" – for two double bonds within their chemical structures). Later there were discovered biologically inactive PGA_2, PGB_2, PGC_2, and biologically active PGD_2 and PGJ_2. These achievements were possible owing to the mass spectrometric techniques, which were highly developed at the Karolinska Institutet. In 1964 David Van Dorp of Unilever in Holland and Sune Bergström of Karolinska Intitutet in Sweden with their coworkers discovered that PGs were biosynthesized from polyunsaturated fatty acids. For the physiologically important PGs of the 2 series – a specific substrate is arachidonic acid (AA) [50]. Incidentally, the homologs of AA: dihomo-γ-linolenic acid (DHLA) and eicosapentaenoic acid (EPA), both may generate *in vitro* PGs of either 1 or 3 series, e.g. PGE_1 or PGE_3 [26]; however, *in vivo* such a conversion of DHLA or EPA to PGs is not sufficiently documented [51].

Rabbit aorta Contracting Substance (RCS), prostaglandin endoperoxides (PPG_2 & PGH_2) and thromboxane A_2 (TXA_2)

The chemical identification of labile prostaglandin endoperoxides (PGG_2 and PGH_2) which serve as direct precursors both for stable prostaglandins and for unstable thromboxane A_2 was preceded by the biological discovery of an unstable "rabbit aorta contracting substance" – RCS. RCS was characterized in the effluent from immunologically challenged perfused lungs of sensitized guinea pigs by Priscilla Piper and John Vane [49] in a bioassay system known as the Vane Bioassay Cascade [60]. The appearance

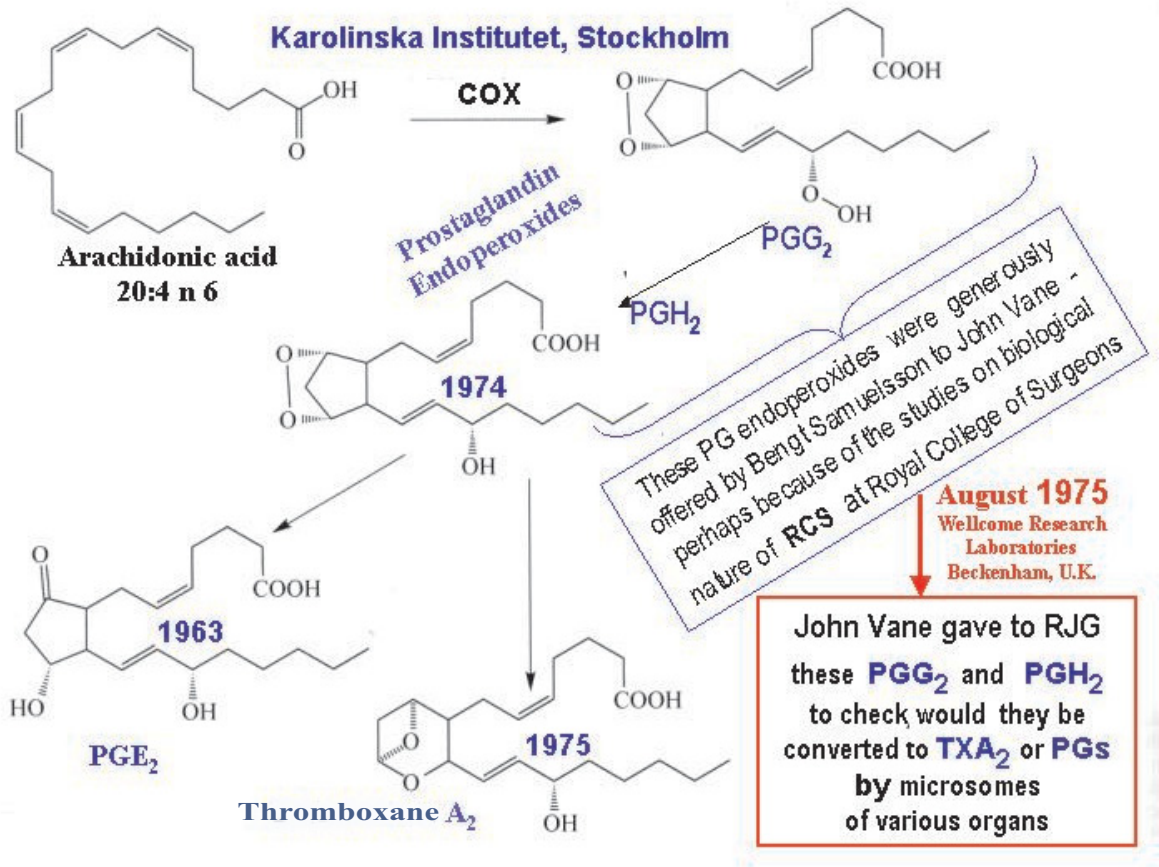

Fig. 1. The saga of the arachidonate family. Biochemical foundation of the saga (in blue) was laid in Stockholm, at Karolinska Institutet by Ulf Svante von Euler, Sune Bergström, Bengt Samuelsson and their colleagues [50]. The Bioassay Cascade of John Vane (Royal College of Surgeons, London and Wellcome Research Laboratories, Beckenham) offered a new opening for continuation of the saga (in red) [32] and eventually for discovering prostacyclin [6, 20, 46, 47]

of RCS was inhibited by aspirin in parallel to the inhibition of the prostaglandin generation [49], as it will be soon announced by John Vane [57]. This discovery [49] was followed by the proposal of the dual nature of RCS by Ryszard Gryglewski and John Vane [32–34]. We found RCS to be generated by tissues not only in a pathological situation such as anaphylactic shock [49]. It is also generated physiologically by spleen slices next to their mechanical stimulation [32, 33] or by splenic microsomes fed with arachidonate [34]. Our splenic unstable RCS only partially behaved like a precursor for stable prostaglandins, and partially it disappeared in thin air without the expected generation of a PG-like material. The biological studies concerning the nature of Rabbit aorta Contracting Substance (RCS) were performed at the Department of Pharmacology of Royal College of Surgeons (RCS) in London (what a coincidence in these abbreviations!). Our studies might have helped at the Karolinska Institutet in biochemical differentiation between unstable prostaglandin endoperoxides (PGG_2, PGH_2) [35, 36] and even more labile thromboxane A_2 (TXA_2) [38] – both discovered by Bengt Samuelsson and his coworkers (Fig. 1).

Prostacyclin appears at the stage

To his last ever published paper John Vane gave the title: "Adventures and excursions in bioassay, the stepping stones to prostacyclin" [56]. Indeed, the unique bioassay technique invented by John Vane – *the Vane Bioassay Cascade* – was the tool that made possible

the discovery of RCS and the discovery of prostacyclin. John McGiff of the Vallhala New York Medical College, the great friend of John Vane depicted *the Vane Bioassay Cascade* as "the triumph of intellect over technology". I would like to add that the versatility of *the Vane Bioassay Cascade* unwillingly introduced elements of hazard into the rigorous laboratory procedures.

No doubt, the foundations for the discovery of prostacyclin were laid by the discovery of RCS in 1969 [49] and our discovery (1971–1972)[32–34] that one part of RCS may serve as a precursor for prostaglandins, while the other disappears spontaneously leaving no biological activity behind itself. The ingenious biochemical studies of Mat Hamberg and Bengt Samuelsson during a period of 1973–1975 proved that the biological activity of RCS would depend partially on prostaglandin endoperoxides (PGG_2 and PGH_2) [36], and partially on thromboxane A_2 (TXA_2) (Fig. 1) [37, 38]. Then Bengt Samuelsson, in a generous gesture, provided John Vane (those days the Research Director of the Burroughs Wellcome Research Laboratories, Beckenham, Kent, UK) with PGG_2 and PGH_2. These endoperoxides were dissolved in anhydrous toluene, sealed, and kept on dry ice. In summer 1975, when I started my sabbatical with John, he handled to **me** these endoperoxides and asked **me** to look for their conversion by microsomes from various organs either to stable prostaglandins (PGs) or to unstable thromboxane (TXA_2) (Fig. 1).

Now, let me change the subject for a while. I brought with me to Beckenham a reprint of our latest paper published in *Prostaglandins* [25], in which we described the discovery of mechanism of action of steroidal anti-inflammatory drugs (e.g. hydrocortisone, dexamethasone). We claimed that glucocorticosteroids act as inhibitors of the release of arachidonate (AA) from cell membranes [25]. The trick was that glucocorticosteroids inhibited the PG generation only in perfused organs with intact cell membranes, but not in their homogenates. This inhibition was waved up in the presence of exogenous arachidonate in the perfusate. Our conclusion was that glucocorticosteroids inhibited the enzymic release of the substrate (AA) from the intact cell membranes but they did not inhibit the enzymic conversion of AA to PGs in a way as aspirin (Fig. 2) and aspirin-like drugs did [12, 57, 60]. John Vane – the discoverer of the mechanism of action of aspirin (Fig. 2) and other non-steroidal anti-inflammatory drugs [58] was strongly critical about our proposal concerning the mechanism of action of steroidal anti-inflammatory drugs. When I refused to repeat our published experiments in Beckenham he asked an excellent biochemist Roderick Flower to check our conception. It ended up with the discovery by Rod Flower of macrocortin – a polypeptide responsible for anti-phospholipase effect of glucocorticosteriods [3]. Subsequently, that peptide link was reshaped to lipocortin and next to a family of annexins, and then the molecular biologists took it over. I mentioned here this story for three reasons. Firstly, it pays off to be stubborn, secondly, even John Vane himself demanded the biochemical confirmation of any conclusion that might emerge from his Bioassay Cascade experiments, and thirdly, to pay the tribute to the unique biochemical skills of Rod Flower. Those were important in the process of discovering prostacyclin.

Let's come back to the main track. Prostaglandin endoperoxides of Bengt Samuelsson sealed in an anhydrous solvent were safely deposited in a refrigerator. The Vane Cascade was ready to be installed. An extremely bright extramural-year student Stuart Bunting was willing to help me. Friendly Roderick Flower performed his biochemical experiments on corticosteroids in an adjacent biochemical section, and we all were working in the pharmacological laboratory directed by Salvador Moncada. With Stuart Bunting we mounted *the Vane Bioassay Cascade* that was equipped with the detectors for PGs (mainly a rat stomach strip, RSS) and for TXA_2 (a special assembly of RbAs capable to differentiate between PG endoperoxides and TXA_2). We found that microsomes from most studied organs converted PG endoperoxides to prostaglandins, exclusively. Only microsomes of the lung and the spleen, not unexpectedly, made some of TXA_2, in addition to PGs. Of course, blood platelets converted PGG_2 or PGH_2 to TXA_2, exclusively. When it came to the pig aortic microsomes – they behaved differently – since neither PGs nor TXA_2 were produced from PGG_2 or PGH_2 by these microsomes, and even worse – our RSS/RbAs cascade detected no biological activity at all. Then we started to use chemical techniques to look for the known products of the non-enzymic decomposition of PG endoperoxides, such as C-17-hydroxy acid (HHT) or malondialdehyde (MDA) [8, 20], however, we were unable to detect either of them. The jokes on "an invisible Polish hormone" appeared in the laboratory. In that situation my *ultimum refugium* was Rod Flower, and indeed, next to the incubation of pig aortic microsomes with

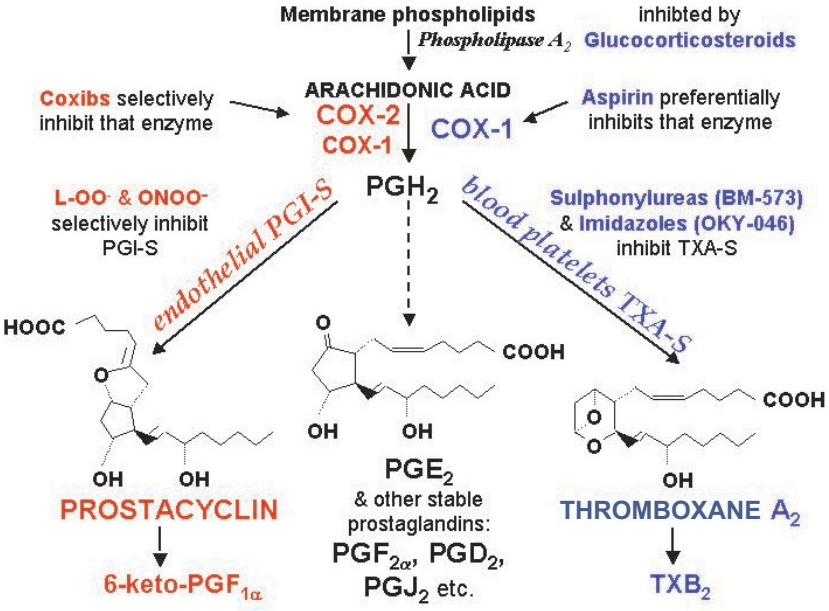

Fig. 2. Pharmacological interference into arachidonic acid cascade. Arachidonic acid (AA) is the substrate for all natural prostanoids. AA is released enzymatically from the membrane phospholipids by phospholipase A_2 (PLA_2). That enzyme is inhibited by natural glucorticosteroids (e.g. hydrocortisone) or by their synthetic analogues (e.g. dexamethasone) [17, 25]. Cyclic endoperoxides PGG_2 and PGH_2 which arise from AA as a result of its enzymic cyclisation by cyclooxygenases: the constitutive COX-1 (e.g. in blood platelets) and the inducible COX-2 (e.g. the endothelial enzyme is induced by shear stress). John Vane with his coworkers discovered the mechanism of pharmacological action of aspirin as an inhibitor of prostahlandin biosynthesis [12, 57, 59]. Nowadays it has become obvious that COX-1 is preferentially inhibited by low doses of aspirin, while COX-2 is selectively inhibited by synthetic coxibs. The COX products, i.e. prostaglandin cyclic endoperoxides (PGG_2 and PGH_2), are isomerised by corresponding synthetases (PGE-S, TXA-S or PGI-S) either to stable prostaglandins (e.g. PGE_2) or to labile thromboxane A_2 (TXA_2) and to labile prostacyclin (PGI_2) [Fig. 2]. Synthetic TXA-S inhibitors like an imidazole derivative OKY-046 or a sulfonylurea derivative BM-573 are tried in prevention of the reperfusion injury next to myocardial ischemia [40]. Aspirin at low doses inhibits platelet COX-1, preferentially [59]. On the other hand PGI-S is inhibited by endogenous linear lipid peroxides (e.g. 15-HPAA) [20, 46] or by peroxynitrite ($ONOO^-$) [61]. The protection of PGI-S from being destroyed by either of those two is a difficult task. Recently, an unusual possibility has been reported that PGI-S may use 15-HPAA as the substrate for generation of a cytoprotective epoxide (13-OH-14,15-EET) [63]. This finding deserves a careful follow up. *In vivo* most anti-oxidants failed to offer a significant protection to PGI-S, however, it is claimed that *via* a specific transport system dehydroascorbate is being translocated from body fluids into endothelial cells. There it is regenerated to ascorbic acid (vitamin C), and that *in statu nascendi* prevents formation of peroxynitrite within endothelial cells – thus protecting PGI-S from being inactivated [42]

PGH_2 he identified in the incubate a biologically inactive prostaglandin, namely 6-keto-$PGF_{1\alpha}$ (Fig. 2). That prostaglandin had been earlier characterized by a Canadian biochemist Cecil Pace-Asciak. At that point the end of our enterprise could have been easily reached. It seemed reasonable to assume that arteries do inactivate highly reactive PG endoperoxides (the precursors of TXA_2 in blood platelets!) to a biologically inactive stabile end product. In that way the arteries, in a self-protective mechanism, would shield themselves from a damage which might be inferred on them by labile, highly reactive, and – therefore – cytotoxic PGG_2, PGH_2 or TXA_2. It was what we were thinking.

Fortunately, we started to play around with our biological detectors within our *Vane Bioassay Cascade*. The strips of rabbit coeliac and mesenteric arteries as well as rat colon were incorporated into the cascade instead of rabbit aortic strips and rat stomach strips which proved themselves to be useless in these new circumstances [6]. Once this important alteration was introduced, we detected a unique set of contractions and relaxation along the cascade (the unknown set of fingerprints as we called it) in response to a mixture of aortic microsomes with PGG_2 or PGH_2 incubated on ice. This biological activity of the newly discovered product disappeared by a half, next to its incubation at 37°C for 4 min. In this way, prostacyclin (PGI_2, those days named by us PGX) was discovered owing to the versatile power of the *Vane's Cascade*. We also found that PGX inhibited blood platelet aggregation, and that PGX might be generated by arterial rings from PG endoperoxides deriving from blood platelets. Moreover, we discovered that endothelial

biosynthesis of PGX was inhibited by linear lipid peroxides [6, 20, 46, 47, 51] (Fig. 2).

The discovery that lipid peroxides are the inhibitors of prostacyclin biosynthesis happened owing to the dry sense of humor of John Vane and because of my laziness. One day when I serenely enjoyed looking at the characteristic "fingerprints" of our PGX appearing along the cascade, John spoiled my pleasure by saying: "Richard, if you really think, that those dances along the cascade which you contemplate with such a delight, represent a new prostanoid supposedly made from PGH_2 by aortic microsomes then you must find an inhibitor of this enzymic reaction". My obvious question was: "how should I look for such an inhibitor ?" The answer was: "I do not know – anything will do – use an alphabetical order". I was cross with him, but perversely I pretended to take his advice seriously and I started my trials with AA – Arachidonic Acid. Usually, the stock solution of AA in anhydrous toluene was stored in an ice-box. When needed potassium salt (K-AA) was prepared *ex tempore* in Krebs' buffer. And so I did. This freshly prepared K-AA had no effect on generation of PGX from PGH_2 by aortic microsomes. Now comes my laziness again – the next day when repeating this experiment instead of preparing a fresh solution of K-AA, I used K-AA in Krebs' buffer that I left overnight on a bench at room temperature. That time, a weak inhibition of PGX generation occurred, however, every next day this inhibition by "K-AA in the buffer" was stronger and stronger. I started to suspect that in the Krebs' buffer a non-enzymatic oxidation of K-AA took place, and that accumulated K-AA peroxides acted as inhibitors of PGX synthase. The Wellcome chemists supplied us with various peroxides prepared *lege artis* from several types of polyunsturated fatty acids, and indeed, all of them including 15-hydroperoxy-arachidonic acid inhibited PGX synthase [20, 46].

Because of those findings we started to think about pathogenesis of atherosclerosis in terms of intoxication with linear lipid peroxides, that would leave arteries without the defensive prostacyclin barrier [15, 16, 19, 20, 28, 30]. This line of reasoning was strengthened, when in a due course we discovered that in cultured endothelial cells (which generate both prostacyclin and nitric oxide (NO) [35]) NO vividly interacted with superoxide anion (O_2^-) [23, 24]. Later it became obvious that in this reaction peroxynitrite ($ONOO^-$) was generated, and this toxic species selectively inhibited the enzymic activity of prostacyclin synthase (PGI-S) [61, 62]. It seems that an excessive activation of NADPH oxidase in blood vessels is mainly responsible for the local generation of vascular reactive oxygen and nitrogen species [10]. A search for the targeted vascular NADPH oxidase inhibitors might be promising.

The prediction of the chemical structure of prostacyclin and its chemical synthesis were relatively easy to accomplish by the high rank chemists. In parallel to the industrial synthesis of PGI_2 by Upjohn Co its chemical structure was correctly recognized on biogenetic grounds by Josef Fried, a professor of Chicago University [44]. As he said it was the second (next to TXA_2) and the last possible chemical structure for an unstable product that might arise by a transformation of PG endoperoxides. Josef Fried synthesized sodium salt of prostacyclin immediately after reading our biological papers on the PGX discovery, and the next year his paper on the first stabile prostacyclin analogue [13] appeared. Josef Fried being an organic chemist had a thorough understanding of Nature, and we owe him a lot in the field of prostanoids. Myself and Andrzej Szczeklik we were the first human beings, who were infused intravenously with prostacyclin [29, 30], and that prostacyclin sodium salt was synthesized by an Italian chemist – Carmelo Gandolfi of Milan.

A hope that dihomo-γ-linolenic acid (DHLA of plant origin) or eicosapentaenoic acid (EPA of fish origin) might be used *in vivo* as the generators of PGI_1 or PGI_3 was not fulfilled [26], although *in vitro* EPA was transformed by the acetone-pentane powder of ram seminal vesicles to PGH_3, that in turn was converted by pig aortic microsomes to Δ-17-6-keto-$PGF_{1\alpha}$ (an expected stable product of PGI_3 decomposition) [51].

Clinical consequences of the discovery of prostacyclin

Nine years after the discovery of prostacyclin we published a book on the clinical trials with prostacyclin [30]. Those days the major issue appeared to be the treatment with intravenous infusions of prostacyclin sodium salt of the patients with peripheral vascular disease. Actually, this line of clinical studies was initiated by Andrzej Szczeklik et al. in 1979 [55]. Critical limb ischemia associated with atherosclerosis, the

Raynaud's syndrome and the Buerger's disease were treated with prostacyclin [30]. Nowadays, when labile prostacyclin sodium salt is replaced by its stable analogues, such as iloprost [27] and a number of the iloprost followers (treprostinil, cicaprost, beraprost, epoprostenol) there appear well documented clinical data pointing out to a high efficacy of the stable prostacyclin analogues for the treatment of advanced critical limb ischemia, especially in the course of Buerger's disease. Out of many reports [27] let me mention only the iloprost beneficial effects during the short- and long-term therapies of critical limb ischemia (the Lariche-Fontaine stages III and IV) [45], reports on beneficial effects in Buerger's disease of another prostacyclin stable analog – treprostinil [11], a report on improvement of endothelial dysfunction by treprostinil in patients with Buerger disease [4], and, finally, a comparison of the therapeutic results obtained in patients with Buerger disease who were subdued either to the lumbar sympathectomy or to the pharmacotherapy with iloprost [5]. The clear-cut message out of this last study is as follows: "In the era of stable prostacyclin analogues there is no reliable evidence to support the use of lumbar sympathectomy in patients with Buerger disease".

Pulmonary endothelium seems to be the major source of prostacyclin in the body [17, 21, 22]. Pulmonary artery hypertension (PAH) is associated with prostacyclin deficiency, and, accordingly, pharmacotherapy of PAH has been dramatically changed owing to the discovery of prostacyclin. The synthetic stable analogues of prostacyclin such as iloprost [40, 41], treprostinil [2, 39], epoprostenol [43], beraprost [48] or cicaprost [14] altered the approach to the therapy of PAH [52], especially when combined with with sildenafil (an inhibitor of phsphodiesterase 5) or bosentan (an antagonist of endothelin. ET-1 receptor).

Finally, there are some well known drugs, which apart from their principal mechanism of action, perform also as "pleiotropic" releasers of endogenous prostacyclin from endothelium [8, 18, 21, 31]. The best known are lipophylic angiotensin converting enzyme inhibitors (ACE-I, e.g. quinapril, perindopril, ramipril) and statins (e.g. atorvastatin) [7, 21, 31]. Therefore, ACE-I which are primarily used for the treatment of patients with arterial hypertension were found by clinicians to prevent the development of atherosclerotic or diabetic or angiopathies. ACE-I not only inhibit generation of a vasoconstrictor angiotensin 2, but they also potentiate endogenous bradykinin that *via* its B_2 receptors is the most potent endogenous releaser of prostacyclin from endothelial cells [35].

Apart from ACE-I the long list of prostacyclin releasers includes statins, some of β-adrenoceptor blocking agents like nebivolol and carvedilol, antiplatelet thienopyridines (ticlopidine, clopidogrel) and anti-diabetic drugs (e,g. gliclazide, metformin) [7, 18, 21, 31]. Stefan Chłopicki et al. [9] recently discovered the prostacyclin – releasing effect of N^1-metylnicotinamide.That one not so long ago was considered to be an inactive product of the metabolism of vitamin PP. In turn, vitamin C (ascorbic acid) showed its new face as a specific intracellular PGI-S protector within endothelial cells. By preventing formation of peroxynitrite ($ONOO^-$) it protects endothelial generation of prostacyclin [42].

John Vane entitled his Croonian Lecture in 1993 at Royal Society: *"The endothelium: maestro of blood circulation"* [58]. At that time he had enough of experimental and clinical data to reach this conclusion. However, the exceptional position of the endothelium in blood circulation was recognised as early as in 1954 by Rudolf Altschul [1], a professor in histology at the University of Saskatchewan in Canada. He was an outstanding researcher and a visionary. He wrote in his book: *"...the secretory function of endothelium need to be considered.... there are reasons to accept its dysfunction in the case of degenerative vascular diseases".* About the personal drama of this great man one may only guess from the dedication printed on the first page of his book : *"To Anni Caroline who was very brave when the ship went down".*

In his book one may come across a microscopic view of the endothelium from arteries at the early stage of atherogenesis. In this particular photograph endothelial cells do not look like ceramic tiles – they look like a palisade of stout but tall, tightly packed grenadiers – ready to defend arterial wall against the invasion. I could not avoid a feeling that Rudolf Altschul had foreseen the existence of prostacyclin synthase, nitric oxide synthase and heme oxygenase in the endothelium – this triad of defenders against atherosclerotic and diabetic angiopathies.

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