Biological activities of non-enzymatic oxygenated metabolites of polyunsaturated fatty acids (NEO-PUFAs) derived from EPA and DHA: New anti-arrhythmic compounds?

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A B S T R A C T

ω3 Polyunsaturated fatty acids (ω3 PUFAs) have several biological properties including anti-arrhythmic effects. However, there are some evidences that it is not solely ω3 PUFAs *per se* that are biologically active but the non-enzymatic oxygenated metabolites of polyunsaturated fatty acids (NEO-PUFAs) like isoprostanes and neuroprostanes. Recent question arises how these molecules take part in physiological homeostasis, show biological bioactivities and anti-inflammatory properties. Furthermore, they are involved in the circulations of childbirth, by inducing the closure of the *ductus arteriosus*. In addition, oxidative stress which can be beneficial for the heart in given environmental conditions such as the presence of ω3 PUFAs on the site of the stress and the signaling pathways involved are also explained in this review.

1. Introduction

The role of omega-3 polyunsaturated fatty acids (ω3 PUFAs) on cardiac disease has been controversial for several decades. Numerous studies have been covered in recent years to elucidate if the ω3 PUFAs have a direct protective effect in cardiac disease (For review see Saravanan et al., 2010; Roy and Le Guennec, 2017). Recently, a new hypothesis has been proposed that involves oxidative stress, and the resulting non-enzymatic oxidation of ω3 PUFAs that can generate bioactive metabolites having cardioprotective properties. Isoprostanoids derived from ω6 and ω3 PUFAs are known biomarkers of oxidative stress in plants and mammals however, in recent studies, some of these derivatives have been shown to hold biological effects. For example, non-enzymatic metabolites of eicosapentaenoic acid (NEO-EPA) and non-enzymatic metabolites of docosahexaenoic acid (NEO-DHA) are responsible of anti-inflammatory properties of EPA and DHA. In this review, we describe known biological activities of these NEO-PUFAs with a particular highlight on their cardioprotective effects and anti-arrhythmic properties. A mechanism of action is proposed on how the ω3 NEO-PUFAs released take part in the prevention of arrhythmias following an ischemic-reperfusion episode. A short description on the underlying biological effects of ω 6 NEO-PUFAs is also noted in this review.

1.1. ω3 PUFAs and cardiac disease

The cardioprotective effects of ω3 PUFAs have been known since the mid-70's (Bang et al., 1976). The intake of fatty fish such as mackerel or tuna was associated with lower risk of cardiac arrhythmias including sudden cardiac death (Burr et al., 1989; Siscovick et al., 1995; Albert et al., 2002) and arrhythmic coronary heart disease (Mozaffarian et al., 2003). Administration of Omacor®, a mixture of 850 mg of eicosapentaenoic acid (C20:5 ω3, EPA) and docosahexaenoic acid (C22:6 ω3, DHA) the two major PUFAs of fatty fish, decreased the incidence of sudden cardiac death in a secondary prevention study of myocardial infarction (GISSI, 1999). Interestingly, in this large randomized clinical trial, the number of myocardial events was not reduced by ω3 PUFAs supplementation but was more effective in reducing the incidence of sudden cardiac death. In fact, sudden cardiac death with consecutive ventricular fibrillation is the major consequence of myocardial

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infarction (Hinkle and Thaler, 1982; Leaf and Hallaq, 1992; Bayes de Luna et al., 1989; Greene, 1990). Recently, the science advisory board of the American Heart Association, Siscovick et al. (2017) carefully analyzed studies of large randomized clinical trials and concluded that among all potential targeted cardiac diseases and cardiac events, only cardiac death was decreased by ω3 PUFA intake. Thereafter, the scientific community concluded that the most probable positive effect of ω3 PUFAs supplementation is by preventing fatal arrhythmias.

1.2. *Anti-arrhythmic effects of ω3 PUFAs*

The precise mechanisms responsible for the anti-arrhythmic properties of PUFAs remain unclear. Intravenous infusion of DHA emulsion in dogs tended to slow heart rate, shortened the corrected QT interval (QTc) at rest and significantly prevented fatal ischemia-induced ventricular arrhythmias (Billman et al., 1999). This experiment confirmed previous reports on the prevention of ischemia-induced ventricular arrhythmias in dogs (Billman et al., 1994) and marmosets (McLennan et al., 1992) by PUFAs. In humans, the consumption of tuna or other fatty fish was associated with a significant slowing of heart rate and reduced likelihood of prolonged QT; the two electrophysiological parameters that can participate in anti-arrhythmic effects of ω3 PUFAs (Mozaffarian et al., 2006). Interestingly, in accordance to Billman et al. (1999), a pilot study on ten patients who were at high risk of sudden cardiac death (Schrepf et al., 2004) showed that the induction of sustained tachycardia was significantly decreased after infusion of a solution containing ω3 PUFAs. This indicates that the effect of ω3 PUFAs acting as anti-arrhythmic agents was viable but it also may need an ischemic period to occur. During myocardial infarction, oxidative stress is induced that contributes to arrhythmias leading to sudden cardiac death (Downey, 1990; Fukuda et al., 2005). Such an abrupt imbalance of the redox status can lead to the oxidation of proteins (Davies, 1987), nucleic acids (Dizdaroglu, 1992) and lipids (Burton et al., 1990). It is widely acknowledged that some of these oxidized products of macromolecules in part could trigger arrhythmias (Tse et al., 2016). For example, Fukuda et al. (2005) showed that an isoketal, E_2-IsoK originating from the non-enzymatic oxidation of arachidonic acid (C20:4 ω6, AA) can form adducts with the sodium channel protein $Na_V1.5$, perturbing its activity in a pro-arrhythmic way.

1.3. *Effect of ω3 PUFAs in cell models*

Experiments performed on isolated cardiac cells suggest that ω3 PUFAs have direct electrophysiological effects (Leaf et al., 2008; Moreno et al., 2012). In a study using isolated cardiac cells of rabbits with heart failure and isolated cells from human heart at the final stage of heart failure (Den Ruijter et al., 2008), the authors showed that the prevention of arrhythmias by ω3 PUFAs (EPA and DHA) was due to reduced diastolic calcium and lower sensitivity of cardiac cells induced by adrenaline. EPA and DHA, the two major ω3 PUFAs found in fatty fishes, hyperpolarized the resting membrane potential and shortened the duration of the action potential (Leaf et al., 2008; Den Ruijter et al., 2008) by modulating the ionic channel activity. An inhibition of the fast sodium current (I_{Na}) was observed in some reports (Xiao et al., 1995; Leifert et al., 1999) and moreover, the ultrafast activation delayed the outward potassium current (I_{KUR}) (Honoré et al., 1994), the inwardly-rectifying rapidly-activated potassium current (I_{KR}) (Guizy et al., 2005), the L-type calcium inward current (I_{CaL}) (Xiao et al., 1997; Verkerk et al., 2006), and the Na^+-Ca^{2+} exchange current (I_{NCX}) (Xiao et al., 2004; Ander et al., 2007) of the membrane. It is also known that ω3 PUFAs blocked the transient outward potassium current (I_{TO}) (Bogdanov et al., 1998; Macleod et al., 1998). It should be noted that, to the best of our knowledge, the ionic current(s) involved in the hyperpolarization induced by ω3 PUFAs has not been clearly identified.

In contrast, it has been shown that NEO-DHA and not DHA *per se*, were active on cardiac ionic channels such as I_{TO} and I_{SS} (Judé et al., 2003). The authors demonstrated this effect by using low concentration of H_2O_2 (1 μM) to favor the oxidation of DHA or α-tocopherol (1 μM) as positive control, and found that the modulatory effects of ω3 PUFAs on cardiac ionic channels were due to NEO-PUFA. Roy et al. (2015) further confirmed this hypothesis in isolated ventricular cells. The authors observed that the anti-arrhythmic potential of DHA was potentiated by pro-oxidants and conversely prevented by anti-oxidants. Another study that showed the possible role of NEO-PUFA in preventing or curing arrhythmias was by Zhao et al. (2012) who showed that in rabbit ventricular cells, early depolarizations induced by high dose of H_2O_2 (200 μM) were inhibited by DHA (10 μM) whereas ROS production by cardiac cells was not changed. In the presence of such concentration of H_2O_2, the 10 μM of DHA in the solution is very likely to oxidize completely suggesting that the anti-arrhythmic effects are not due to DHA itself but due to NEO-DHA generated from DHA oxidation.

2. Oxygenated metabolites of ω3 PUFAs

PUFAs are vulnerable substrates for enzymatic and non-enzymatic oxidative transformations. Collectively, these metabolites formed are known as oxylipins. The oxidative transformations are due to the presence of multiple double bonds in their structures, and more so in the presence of two double bonds surrounding a methylene group (-CH = CH-**CH_2**-CH = CH-). Such configuration creates weak methylene C-H bond and readily allow H-abstraction by heme (cyclooxygenases) or non-heme (lipoxygenases) enzymes, iron-containing enzymes (enzymatic transformation) or free radical species (non-enzymatic transformation). Enzymatic H-abstraction of the substrate is very specific with regard to the position of the methylene bond whereas the abstraction is random for non-enzymatic oxidative transformations and consequently the ensuing product profile is more complex and more so, when the number of double bonds in the PUFA increases. Of note, the radical mechanisms following H-abstraction are the same for enzymatic and non-enzymatic oxidation. For example, in Fig. 1A, the H-atom of AA is abstracted at the 13th position by cyclooxygenases or 15-lipoxygenase (15-LOX) or by ROS. The resulting carbon radical (compound **A**), in the presence of oxygen, further proceeds to form a peroxyl radical (compound **B**) which react with another molecule of PUFA (AA for example) to produce hydroperoxide products i.e. 15(S)-hydroperoxyeicosatetraenoic acid (15(S)-HpETE via 15-LOX) or racemic 15-HpETE (via ROS) and another carbon-centred radical (compound **A**), hence ensuing the propagation step needed for auto-oxidation processes. In the case of enzymatic processes, it is the production of hydroperoxide that oxidizes the heme or non-heme iron-containing enzymes and further pursuits the enzymatic auto-oxidation.

Another fate of peroxyl radical **B** is the formation of an endoperoxide carbon centred radical (compound **C**) which then proceeds through a cyclization step to form the cyclopentane ring followed by final oxygenation to produce **G_2-IsoP** or **PGG_2**. This second consecutive 5-*exo*-trig cyclization of compound **C** follows the models of Stanley and Pryor, and of Funk and Porter (structures **C^1** and **C^2**) which are the origin of the main structural differences between prostaglandins (PG) and Isoprostanes (IsoPs) e.g. 1,2 lateral chains relationship is predominantly *cis* configuration for IsoP, while the enzymatic 3D constrain model (structure **C^3**) prefers the *trans* configuration only. Finally, the reduction of the peroxide and endoperoxide groups gives the isoprostanoids 15-F_{2t}-IsoP and 15-F_{2c}-IsoP that is considered a large part of the oxylipins.

Neuroprostanes (NeuroPs) is derived from DHA and the formation does not involve cyclooxygenases and instead inhibits them (Ringbom et al., 2001). Consequently, NeuroPs only exist via a non-enzymatic process (Roberts et al., 1998), and independent from enzymatic oxidation. Therefore, these are very much unique metabolites of DHA. As described in Fig. 1B, eight different series of NeuroP can be generated via free radical mechanism.

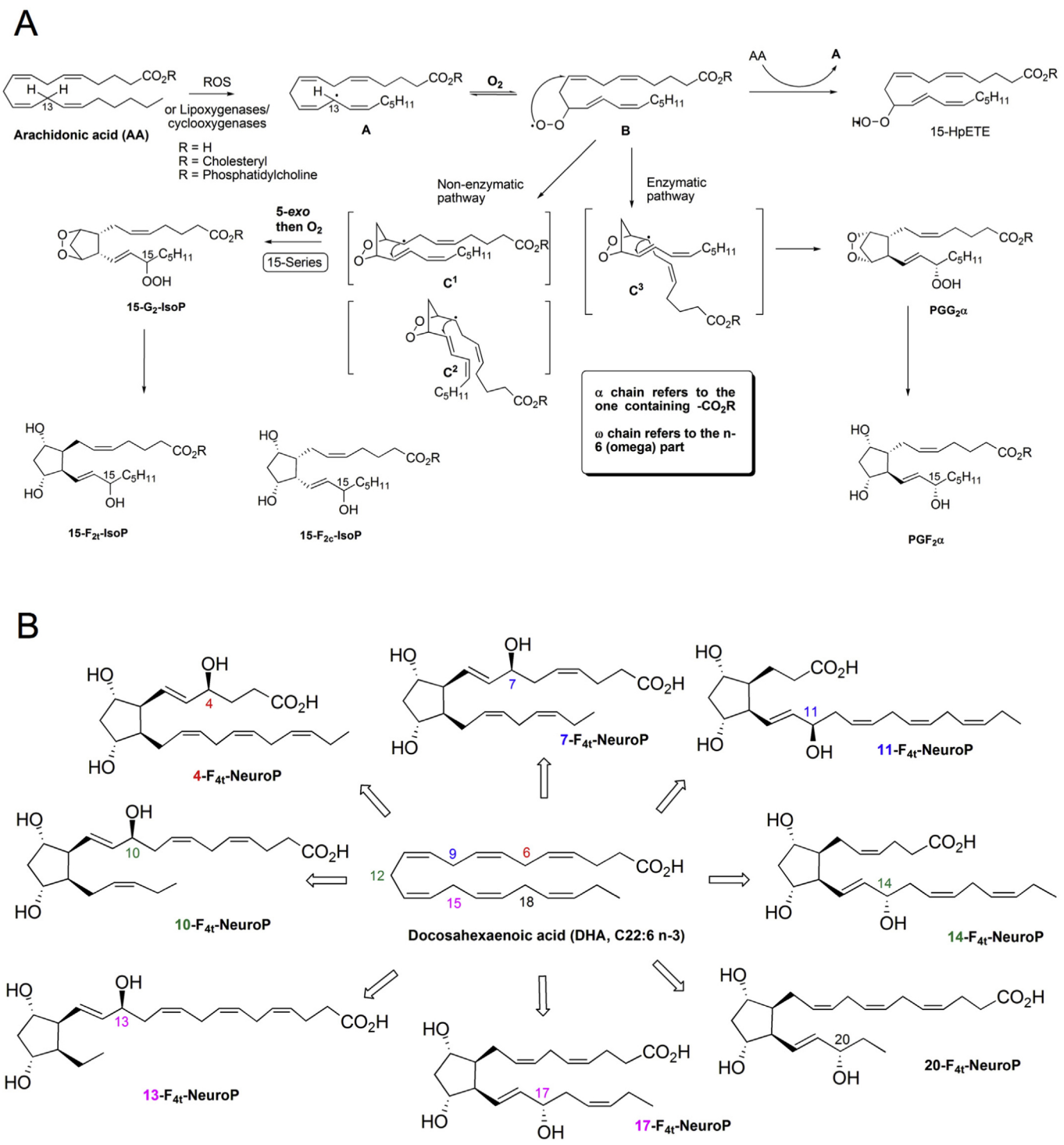

Fig. 1. Chemistry of isoprostanoids. **A)** Biosynthesis of prostanoids and isoprostanoids (only the 15 series of isoprostanoids are displayed). **B**) Representation of the eight possible neuroprostanes formed from DHA non-enzymatic peroxidation.

3. Biological activities of NEO-PUFAs

3.1. Physiological roles of NEO-PUFAs in non-cardiac functions

NEO-PUFAs are considered as biomarkers of oxidative stress (Van't Erve et al., 2017) but recent studies showed them to exert physiological functions. A remarkable point to notice about isoprostanes (IsoPs) and other NEO-PUFAs is that, they are often assumed to be inherently deleterious (Chen et al., 2013; Njie-Mbye et al., 2013; Bauer et al., 2014; Gauthier et al., 2014; Spinelli et al., 2014) particularly, F_2-IsoPs from AA. However, F_3-IsoPs from EPA is considered to be biologically inactive, but high consumption of fish elevated blood F_3-IsoPs and suppressed F_2-IsoPs leading to cardioprotection in Canadian Inuit population (Alkazemi et al., 2016). The authors explain the cardioprotection afforded by ω3 PUFAs in fish was due to competition between ω3 PUFAs and AA in the phospholipids leading to decreased plasma concentration of deleterious F_2-IsoPs. Nevertheless, a careful analysis of the literature suggests that it is not so simple (Fig. 2).

In the resolution of inflammation, it appears more and more obvious that NEO-PUFAs from ω3 PUFAs are likely to play a unique role that is

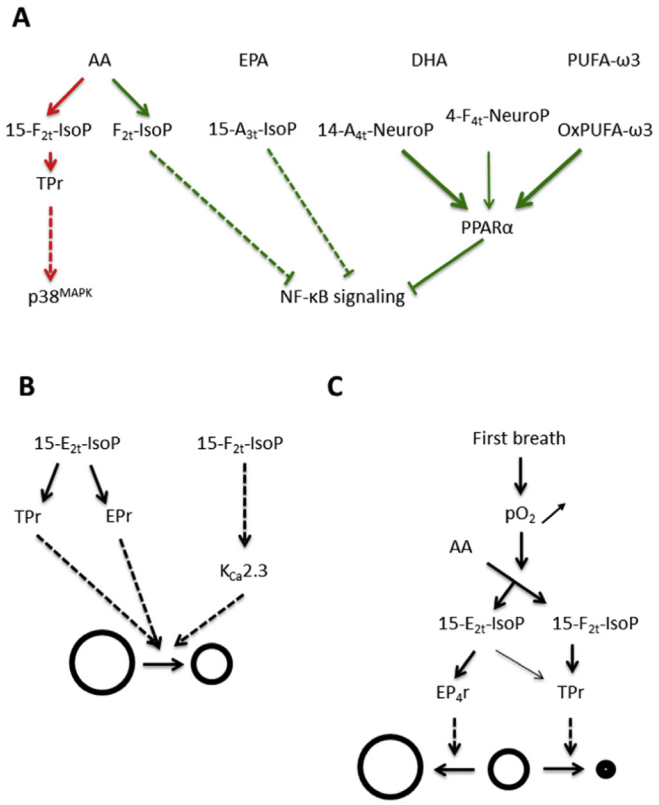

Fig. 2. Known signaling pathways of IsoP and NeuroP from arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Dotted lines suggest that there are unknown intermediate pathways while the others can be direct. **A**) Anti- and pro-inflammatory effects of IsoPs and NeuroPs. The red lines indicate pathways involved in pro-inflammatory situations while those in green indicate the anti-inflammatory pathways. **B**) Signaling pathways involved in the vasoconstrictive properties of IsoPs. TP and EP are prostanoid receptors of T and E type respectively. $K_{Ca}2.$, potassium channel activated by intracellular calcium. When activated, the channel induces the depolarization of the smooth muscle cell membrane leading to vasoconstriction. **C**) Signaling pathways involved in the closure of the *ductus arteriosus* at birth. During the first breathe, the blood pO_2 increases that lead to the production of IsoPs like 15-E_{2t}-IsoP and 15-F_{2t}-IsoP and interact with the prostanoid receptors EP_4r and TPr. The activation of EP_4r produces a dilatation of the *ductus arteriosus* while the activation of TPr is vasoconstrictive. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

at least comparable to anti-inflammatory resolvin E, which are metabolites of EPA released through enzymatic oxidation. Evidently, increased consumption of ω3 PUFAs reduced chronic inflammation and the incidence of consecutive disorders such as atherosclerosis (Allaire et al., 2016; Gladine et al., 2014; Calder, 2015). Sethi's group (Sethi et al., 1996, 2002; Mishra et al., 2004) first corroborated that oxidized EPA and DHA and not native EPA or DHA possessed anti-inflammatory properties. The same authors further confirmed that EPA and DHA need to be oxidized to activate PPARα and in turn, could inhibit NF-κB activation, a transcription factor regulating the expression of pro-inflammatory molecules to activate endothelial cells for binding to monocytes (Sigal, 2006). Thereafter, it has been shown that NEO-PUFAs were able to form ligands with PPARα as well as PPARγ (Itoh et al., 2008).

Regardless to Sethi's group observation, it is of interest to note Brooks et al. (2011) report. The group identified that 15-A_{3t}-IsoP, another NEO-EPA, was also a potent anti-inflammatory molecule by inhibiting NF-κB in macrophages. More recently, a comparison of the putative anti-inflammatory effects of protectin (NPD1 and PDX, metabolites of enzymatic oxidation of DHA) and neuroprostanes (14-A_{4t}-NeuroP and 4-F_{4t}-NeuroP, NEO-DHA) on human macrophages were performed for the first time (Bosviel et al., 2017). The authors showed that the most active metabolite to reduce the levels of cytokines IL-6 and TNF-α through activation of PPARγ and not PPARα, was 14-A_{4t}-NeuroP. However, 4-F_{4t}-NeuroP activated PPARγ albeit with less potency. The authors concluded that, depending on the concentration of the metabolites, protectins and neuroprostanes have similar potency and likely to have complementary anti-inflammatory properties. Moreover, it should be mentioned that 14-A_{4t}-NeuroP was indeed previously discovered to be anti-inflammatory via the inhibition of the NF-κB pathway (Musiek et al., 2008).

Leitinger et al. (2001) found that 15-F_{2t}-IsoP (also known as 8-iso-$PGF_{2\alpha}$), a NEO-AA, stimulated human endothelial cells-monocytes interaction independently and not through NF-κB pathway. It is of interest the finding by Kumar et al. (2005) who showed that the properties of NEO-AA depended on the type of vascular system. In large vessels such as aorta and umbilical vein, 15-F_{2t}-IsoP was found to be pro-inflammatory through the stimulation of endothelial cell-monocytes interaction whereas in the microvasculature, the primary site of inflammation, showed exactly the opposite effect. The group explained the effect may occur through two different pathways. One pathway involves a TP receptor and the other one, an unknown receptor. Similar inhibition of NF-κB and monocyte-endothelial cell adhesion was also observed in porcine pulmonary arteries by another neuroprostane, A_4/J_4 NeuroP (Majkova et al., 2011). To date, the receptors involved and the associated signaling pathways have not been elucidated. Moreover, F_2-IsoPs showed to increase cell proliferation and endothelin-1 release through an unknown receptor coupled to a phosphoinositide pathway (Yura et al., 1999). Such effect was observed in the development of a hepatorenal syndrome that is characterized by intense vasoconstriction.

Beside the anti-inflammatory properties of NEO-PUFAs, another physiological role of these compounds emerged: regulation of vascular tone (Cracowski and Durand, 2006). A thromboxane A2 (TXA_2) mimetic, U-46619, and an IsoP, 15-E_{2t}-IsoP (noted as 8-isoPGE_2 in the report) showed to regulate the human placental vascular tone and resistance (Hausermann and St-Louis, 2011). This role appeared to be important since the placenta was devoid of autonomic innervation and nitric oxide (NO) regulation was inefficient. The effects of both metabolites of AA, enzymatic (TXA_2) and non-enzymatic (15-E_{2t}-IsoP), were mediated through prostanoid receptors, TP and EP respectively.

Angiotensin II (AngII) is a potent constrictor of the aorta and mesenteric arteries. Pfister et al. (2011) showed that AngII-induced vasoconstriction of these vessels was partly due to the production of superoxide and in turn, generated a F_2-IsoP that is not 15-F_{2t}-IsoP. Interestingly, this finding was also mentioned in vascular disease where the production of superoxide is a physiological event. For example, it has been shown to occur in β-adrenergic receptor activation and recognized that the production of superoxide is needed for the physiological activation of the β-adrenergic signaling pathways (Andersson et al., 2011; Llano-Diez et al., 2016) and probably, the same applies to the AngII pathway (Pfister et al., 2011). Gauthier et al. (2014) also found that 15-F_{2t}-IsoP can induce the constriction of middle cerebral arteries through the inhibition of $K_{Ca}2.3$ potassium channels. However, the authors did not investigate whether this inhibition is consecutive to any prostanoid receptors.

The most interesting physiological role of isoprostanes is its participation in the closure of the *ductus arteriosus* of newborn. The *ductus arteriosus* is a vascular shunt between pulmonary artery and the aorta that remains open during fetal life, and constricts in the hours after birth. This closure allows the redirection of blood flow from the fetal gas exchange organ to the placenta and to the newly inflated lungs. The *ductus arteriosus* closure is mediated in part by increased oxygen tension that can activate ionic channels and the withdrawal of vasodilatory prostaglandins. Van der Sterren and Villamor (2011) and Chen et al. (2012) showed that 15-E_2-IsoP and 15-F_2-IsoP were potent vasoconstrictors of the *ductus arteriosus* in chicken and mouse, and the TP

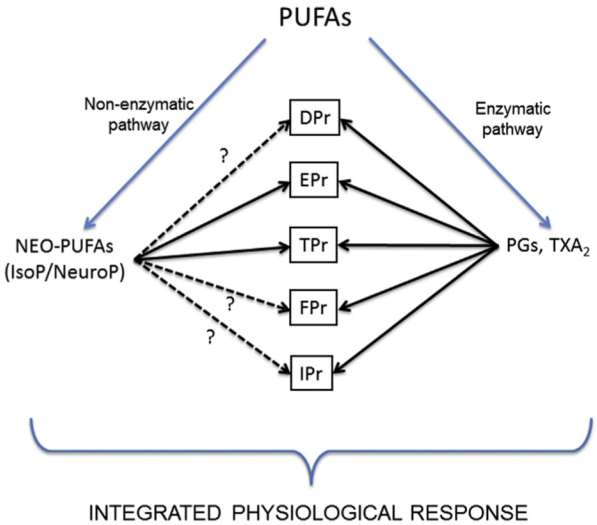

Fig. 3. NEO-PUFAs can activate prostanoid receptors of prostaglandins and thromboxane A2. This has been shown for EPr and TPr (plain lines) but not studied for the other receptors (dotted lines). The cross-talk between the three kinds of stimulators: NEO-PUFAs, enzymatic metabolites and prostanoid receptors needs to be determined to understand the physiological response.

receptors were responsible to mediate these effects. They proposed that the sharp transition from the relatively low oxygen intrauterine environment to a higher oxygen extrauterine environment exposes the newborn to an oxidative challenge. This induces the production of IsoPs that participates to the *ductus arteriosus* closure and the separation of the two blood circulations. These findings are of interest since they showed that a change in environmental pO_2 can be signaled for NEO-AA to "read" and subsequently produce IsoPs in response to physiological reaction. Chen et al. (2012) also showed that 15-E_{2t}-IsoP can activate EP_4r that evokes the relaxation of *ductus arteriosus* where EP_4r are predominant from mid-gestation and shift the TP receptor expression predominantly at gestation term.

IsoPs derived from AA and EPA have been shown to interact with some selectivity with prostanoid receptors like TP and EP concomitantly with enzymatically formed metabolites suggesting important physiological interactions between those enzymatic and non-enzymatic metabolites of PUFAs (Jamil et al., 2012, 2014; Njie-Mbye et al., 2013; Bosviel et al., 2017) (Fig. 3).

3.2. NEO-PUFAs and arrhythmias

Numerous reports on cardiac anti-arrhythmic effect by DHA and EPA, especially following a cardiac stroke are found (for reviews see Siscovick et al., 2017). Based on the work of Judé et al. (2003), the question was left open on whether DHA (and EPA) or NEO-DHA (and NEO-EPA) exerted anti-arrhythmic properties. To answer the question, Roy et al. (2015) tested the anti-arrhythmic properties of DHA in different oxidative environments (with or without 1 μM H_2O_2 or α-tocopherol) in isolated cardiac cell model and arrhythmic mice model. In both models, the group found that DHA needs to be oxidized to exert anti-arrhythmic property. Natural F_4-NeuroPs that was chemically synthesized were tested, and it was found 4(*RS*)-4-F_{4t}-NeuroP (4-F_{4t}-NeuroP) was the most active anti-arrhythmic molecule with IC_{50} approximately 100 nM. In certain condition, the type 2 sarcoplasmic reticulum (SR) calcium channel ryanodine receptor (RyR2), does not close perfectly leading to calcium leak from the SR and consequently cause arrhythmias (Marks, 2001; Beccera et al., 2016). This calcium in turn activates the sodium-calcium exchange that produces an arrhythmogenic inward current (I_{Ti}). The leaky behavior of RyR2 was due to abnormal phosphorylation, S-nitrosylation and/or carbonylation leading to uncoupling of RyR2 with the regulatory protein FKBP12.6 (Fauconnier et al., 2010). In the presence of 4-F_{4t}-NeuroP, phosphorylation, S-nitrosylation and carbonylation were reduced and arrhythmic events were lessened (Fig. 4).

This study underlines the role played by a specific NEO-PUFA, here a NEO-DHA, on cardiac arrhythmias as the other NeuroPs tested by Roy et al. (2015) had lower activity. However, it is not clear whether 4-F_{4t}-NeuroP interacts directly with the RyR2 or with receptors, such as prostanoid receptors, that are responsible to reduce post-translational modifications (Narumiya et al., 1999). This discovery can explain some contradictory results (for a review on this topic, see Billman, 2013) obtained with ω3 PUFAs and opened a new field in the search for new endogenous anti-arrhythmic molecules.

Indeed, in animal and cell experiments, most of the studies on anti-arrhythmic effects byω3 PUFAs have been performed without taking

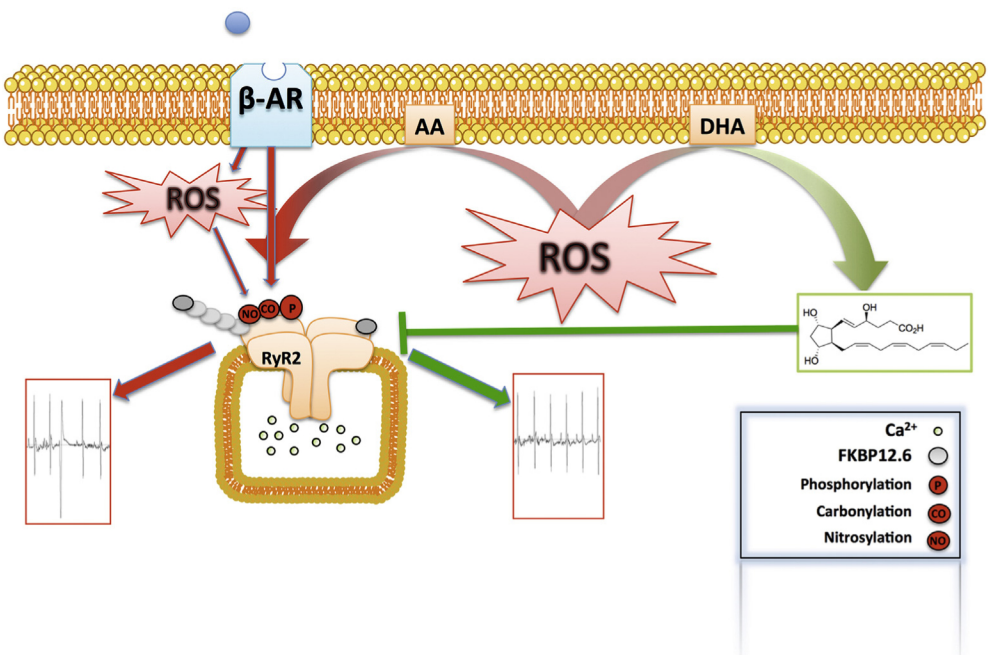

Fig. 4. Schematic diagram explaining signalization pathways involved in the anti-arrhythmic effects of 4(*RS*)-4-F_{4t}-NeuroP. Sympathetic activation and ROS peroxidize molecules like arachidonic acid that will carbonylate, S-nitrosylate and phosphorylate the RyR2 calcium channel inducing a destabilization of the complex RyR2-FKBP12.6. Subsequently, the channel complex becomes leaky and provoke arrhythmias. The 4(*RS*)-4-F_{4t}-NeuroP, prevent/reduce carbonylation/S-nitrosylation/phosphorylation of the RyR2, and prevent the destabilization of RyR2/FKBP12.6 complex hence reduce calcium leakage from the sarcoplasmic reticulum and thus arrhythmias.

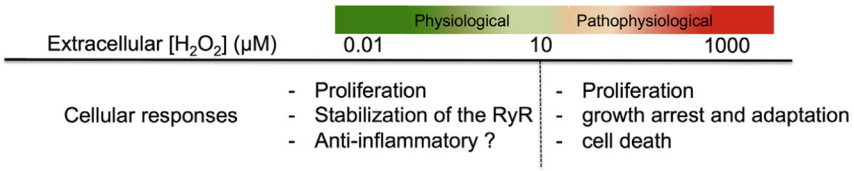

Fig. 5. Cellular response to external H_2O_2. Depending on the concentration of H_2O_2 in the environment surrounding cells, the response of the cells can be physiological (below 10μ M) or pathophysiological (above 10 μM). A "?" is put after *anti-inflammatory* since there are no direct evidence of a link between H_2O_2 and the anti-inflammatory properties of ω3 PUFAs even if it is known that anti-inflammatory IsoPs must be produced in such conditions. Adapted from Stone and Yang (2006).

into account the development of PUFA peroxidation. Some experiments in dog and humans were designed to test the direct effects of ω3 PUFAs by intravenously injecting an emulsion of EPA and DHA (Billman et al., 1999; Schrepf et al., 2004; Den Ruijter et al., 2008). It is very likely that the emulsion of ω3 PUFAs auto-oxidized to NEO-PUFAs *in vivo* in such experimental conditions, and accounted for the observed effects. However, the quality of the emulsion should be eminent as ω3 PUFAs are prone to peroxidation and may be loaded with NEO-PUFA therefore, precautions must be taken in preparing emulsion of oils containing ω3 PUFAs, including supplemental pills which has been underlined recently (Albert et al., 2015).

4. Oxidative stress or oxidative signal?

The increasing number of publications on the biological effects of NEO-PUFAs like F_4-NeuroPs should decipher the question on its physiological and not pathophysiological role of oxidative stress in diseases. Classically, oxidative stress is understood as disequilibrium between oxidants and antioxidants that create excessive ROS. ROS is often used as a generic term describing deleterious molecules that can oxidize proteins, lipids and nucleic acids resulting in disastrous consequences in most of the time. This dogma leads to ignore papers that described the importance of ROS in regulating cardiac function of the β-adrenergic signaling pathway. The inotropic response observed with β-stimulation is due to the production of ROS by mitochondria (Andersson et al., 2011). Acutely, this is beneficial for the heart, and chronically it could become deleterious. However, in some cases, like in the metabolic syndrome, there is no production of ROS with the β-stimulation suggesting an adaptation to avoid a potentiation of the chronic oxidative stress (Llano-Diez et al., 2016). Knowing the importance of the autonomic nervous system in the prevention/triggering of arrhythmias (Champéroux et al., 2015), the beneficial and deleterious effects of ROS when the sympathetic system is activated needs to be more precisely defined. Therefore oxidative stress should be advocated as an event where a transient or permanent perturbation in the ROS balance-state generates physiological consequences within the cell, for which the precise outcome depends on ROS targets and concentrations. ROS refers to four molecules mainly, superoxide, hydrogen peroxide, peroxynitrite and hydroxyl radicals. Among them, one in particular, hydrogen peroxide (H_2O_2) is the product of superoxide dismutase (SOD) and NADPH oxidases (NOX), and metabolized in water by catalases and peroxidases and thereof enzymatically regulated (see Stone and Yang, 2006). The intracellular physiological concentration of H_2O_2 can vary from 0.001 to 0.7 μM in response to a signal (Stone and Yang, 2006). It has been shown to retard aging by oxidizing peroxiredoxins giving a new role for H_2O_2 signaling in proteostasis and lifespan control (Hanzén et al., 2016). It is of important to note that the oxidation of proteins by H_2O_2 is an intermediate step in the physiological signaling (Rhee et al., 2018). In the light of this review, it is proposed that IsoPs and NeuroPs are intermediate lipid mediators in H_2O_2 physiological signaling pathway suggesting that such oxylipins do matter as much as other oxylipins including the eicosanoids.

H_2O_2 is produced in high concentrations by macrophages (respiratory burst) to kill bacteria but it is also a metabolite of the superoxide anion via superoxide dismutase (SOD). H_2O_2 is also produced in wound at low concentrations. Its removal slows down the healing process suggesting that it is necessary for healing to efficiently take place (Roy et al., 2006; Loo et al., 2012). Consequently, a mild oxidative stress involving H_2O_2 could be a participating signal for physiological cellular homeostasis. This proposal is not completely new since Stone and Yang (2006) proposed that H_2O_2 could be a signaling messenger that stimulates biological responses and activates specific biochemical pathways. However, the role as a messenger occurs only at micromolar concentrations range. Indeed the authors proposed that intracellular concentrations of H_2O_2 up to 1 μM (occurring when the extracellular concentration is 10 times higher, 10 μM) lead to physiological cellular responses like proliferation (Stone and Yang, 2006; Roy et al., 2017). This implies that up to 10 μM, H_2O_2 is physiological and the reactions it provokes are normal. The deleterious effects occur at higher concentrations that correspond to the oxidative stress *per se* (Fig. 5).

In the heart, this hypothesis needs to be specifically addressed by using mice conditionally knock-out for superoxide dismutase and/or NADPH oxidase in order to evaluate their cardiac response to myocardial infarction as well as the regulation of the cardiac cycle by sympathetic activation. The same kind of experiments could be performed on mice over-expressing superoxide dismutase and/or NADPH oxidase or catalase. Mice over-expressing SOD1 have been developed and studied a decade ago (Thireau et al., 2008), but this animal model was not conditional and above all, the consequences of a myocardial infarction after cardiac cell membrane PUFAs enrichment have not been studied. At least, it must be underlined that they have a cardiac phenotype reproducing some aspects of the cardiac phenotype found in patients having Down syndrome.

5. Summary and perspectives

In the light of the recent knowledge about the physiological role of H_2O_2 and NEO-PUFAs, we propose that the anti-arrhythmic effects of ω3 PUFAs present in phospholipids (Friedli and Freigang, 2017) are due to their oxidation by H_2O_2 produced consecutively to the cardiac infarct that in turn metabolized to NEO-PUFAs such as F_3-IsoPs and F_4-NeuroPs. These NEO-PUFAs will subsequently reverse or prevent the phosphorylation and carbonylation of RyR2, stabilizing its interaction with FKBP12.6 and normalizing the channel function and thus stopping arrhythmias (Fig. 4).

There are probably some other roles of the NEO-PUFAs but the research field has only opened recently and an easier access to the signaling molecules will help to advance in the understanding of their physiological role. Fully understanding the role of NEO-PUFAs will clarify their interaction with enzymatic eicosanoids such as prostaglandins and enable to fine-tune the mechanistic action of ω3 PUFAs.

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