**HYPOTHESES**

**Endothelium-dependent contraction: The non-classical action of endothelial prostacyclin, its underlying mechanisms, and implications**

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**Funding information**
National Natural Science Foundation of China (NSFC), Grant/Award Number: 31771272, 81970433, 81770678 and 82070506; Natural Science Foundation of Guangdong Province, Grant/Award Number: 2019A1515011650

**Abstract**

Although commonly thought to produce prostacyclin (prostaglandin I₂; PGI₂) that evokes vasodilatation and protects vessels from the development of diseases, the endothelial cyclooxygenase (COX)-mediated metabolism has also been found to release substance(s) called endothelium-derived contracting factor(s) (EDCF) that causes endothelium-dependent contraction and implicates in endothelial dysfunction of disease conditions. Various mechanisms have been proposed for the process; however, the major endothelial COX metabolite PGI₂, which has been classically considered to activate the I prostanoid receptor (IP) that mediates vasodilatation and opposes the effects of thromboxane (Tx) A₂ produced by COX in platelets, emerges as a major EDCF in health and disease conditions. Our recent studies from genetically altered mice further suggest that vasomotor reactions to PGI₂ are collectively modulated by IP, the vasoconstrictor Tx-prostanoid receptor (TP; the prototype receptor of TxA₂) and E prostanoid receptor-3 (EP3; a vasoconstrictor receptor of PGE₂) although with differences in potency and efficacy; a contraction to PGI₂ reflects activities of TP and/or EP3 outweighing that of the concurrently activated IP. Here, we discuss the history of endothelium-dependent contraction, evidences that support the above hypothesis, proposed mechanisms for the varied reactions to endothelial PGI₂ synthesis as well as the relation of its dilator activity to the effect of another NO-independent vasodilator mechanism, the endothelium-derived hyperpolarizing factor. Also, we address the possible pathological and therapeutic implications as well as questions remaining to be resolved or limitations of our above findings obtained from genetically altered mouse models.

**KEYWORDS**

EDCF, endothelial COX, EP3, IP, PGI₂, TP

**Abbreviations**: AA, arachidonic acid; ACh, acetylcholine; Ang II, angiotensin II; COX, cyclooxygenase; EDCF, endothelium-derived contracting factor; EDHF, endothelium-dependent hyperpolarizing factor; EP3, E prostanoid receptors 3; EP3−/−, EP3 deficiency; IP, I prostanoid receptor; L-NAME, N⁰-nitro-L-arginine methyl ester; NOS, NO synthase; PE, phenylephrine; PGI₂, prostaglandin I₂; PGIS, PGI₂ synthase; PLA₂, phospholipase A₂; SHR, spontaneously hypertensive rat; TP, Tx-prostanoid receptor; Tx, thromboxane; WKY, Wistar-Kyoto rat.

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1 | INTRODUCTION

1.1 | Cyclooxygenase-catalyzed metabolism, the classical action of prostacyclin, and the balancing theory

The metabolism of arachidonic acid (AA) via cyclooxygenase (COX; existing in COX-1 and COX-2 isoforms) plays an important role in regulating cardiovascular function. Specifically, COX metabolizes AA released from the membrane lipid pool by phospholipase A2 (PLA2) to form an intermediate PGH2 that is further converted by specific synthases or non-enzymatic isomerization into biologically active end-products, including thromboxane (Tx) A2, prostacyclin (prostaglandin I2; PGI2), PGE2, PGF2α, and PDG2, which are proposed to act on a specific receptor or a subgroup of receptors.1-6 In the circulatory system, COX mainly exists in platelets and the vascular endothelium where AA metabolism produces TxA2 (COX-1) or PGI2 (COX-1 and -2, but the former appears to be the major form in mice) respectively. TxA2 and PGI2 are shortly hydrolyzed into biologically inactive metabolite TxB2 or 6-keto-PGF1α (half-lives: 30 s for TxA2, while 3 min for PGI2), which is then further disposed in the liver and kidneys.4,5,7-11

TxA2 activates the Tx-prostanoid receptor (TP) to mediate platelet aggregation and cause vasoconstriction. On the contrary, PGI2 is classically considered to act on the I prostanoid receptor (IP), which evokes vasodilatation and opposes the effects of TxA2 produced in platelets. The effects of TxA2 and PGI2 are proposed to be balanced; a decrease in PGI2 or an increase in TxA2 could result in the development of vascular pathologies, such as hypertension and atherosclerosis.1-6 This concept is the foundation, on which a low dosage of aspirin (which is suggested to selectively inhibit COX in platelets) is prescribed for patients to prevent the development of diseases, such as atherosclerosis and ischemic cardiovascular disorders.12,13

1.2 | Endothelium-dependent contraction and common explanations

Although the above theory regarding the platelet COX pathway remains valid,13 that related to the vascular endothelium has been challenged by findings of many studies even not long after it was proposed. About 4 decades ago, Dr Paul Vanhoutte’s group showed that in some arteries and venous tissues, the COX substrate AA or agonists, such as acetylcholine (ACh) and thrombin, evoke contractile activity that is endothelium- and COX-dependent.14,15 They further found that these contractions were frequently observed under pathological or disease conditions, and were inhibited by PLA2 inhibition or TP antagonism.16-18 It was proposed that under disease conditions, the endothelial COX can also produce TxA2 and the intermediate or its precursor PGH2 (which also acts on TP) to act as endothelium-derived contracting factors (EDCFs) that evoke vasoconstrictor activity and play an important role in the development of endothelial dysfunction.16,19-22

Also, there are studies suggesting that superoxide or other reactive oxygen species act as EDCFs.23,24 However, it has later been pointed out that superoxide or other reactive oxygen species are more likely to act via decreasing the bioavailability of NO or stimulating the production of vasoconstrictor prostanoisds including TxA2 and PGI2, which links the release of EDCFs to pathological conditions.25-32 In addition, there are studies suggesting that other vasoconstrictor prostanoisds, such as PGE2 and PGF2α act as EDCFs.33-37 Moreover, it has been suggested that under disease conditions, a decrease in the activity of PGI2 synthase (PGIS) caused by tyrosine nitration or an up-regulation of COX-2, which is coupled to PGIS to a lesser extent compared to COX-1 and could also be caused by an increase in the production of superoxide or other reactive oxygen species, can result in a shift in the prostanoisd profile away from PGI2 and towards the production of vasoconstrictor prostanoisds, including TxA2.38-44 Besides, the endothelium may also release endothelin-1, a peptide that causes vasoconstriction and facilitates the release of prostanoisds; however, the release of the peptide is not related to the acute stimulation with agonists, such as ACh, and hence is not discussed here.45,46

Meanwhile, in many vascular beds including those of diseases, COX-1 remains as the major COX form in the endothelium and the production of PGI2 in the vasculature has been seen not to decrease but to increase, rendering the prostanoisd to remain as the predominant COX product produced by the vascular endothelium.7,47-53 The ineffectiveness of its synthase inhibitors along with the inability to increase after agonist stimulation suggests that TxA2 is not a significant EDCF.34,46,54 Also, the role of PGH2 might be questionable, since the compound can automatically convert to prostanoisds other than TxA2 and PGI2 in aqueous conditions that also activate TP, although it alone has been reported to evoke vasoconstriction in vitro as well.47,55,56

2 | PGI2’S EMERGING AS A MAJOR EDCF AND PROPOSED MECHANISMS FOR THIS NON-CLASSICAL ACTION

2.1 | The non-classical action of endothelial PGI2 and its being recognized as an EDCF in disease conditions

The work of Rapoport RM and Williams SP more than two decades ago showed that in aortas of adult Wistar-Kyoto
or spontaneously hypertensive rats (WKYs or SHRs respectively) the endothelium-dependent contraction to ACh could not be completely inhibited by TP antagonism, which was however able to completely abolish a contraction to the TxA₂ analogue U46619. In addition, they found that in the vessels ACh evoked a production of PGI₂, which was even higher in SHRs than in WKYs. Moreover, the authors found that PGI₂ analogues evoked vasoconstrictor responses in these vessels even after TP antagonism and then suggested that PGI₂ may also mediate endothelium-dependent contraction, which was in contrast to the classical action of endothelial PGI₂ commonly believed. Indeed, PGI₂ itself had been repeatedly reported earlier by many groups to have a vasoconstrictor response; however, such a response had been commonly suggested to be sensitive to TP antagonism, usually of a subtle extent, and obtained at higher concentrations than those of PGI₂ to evoke vasodilatation.

Interestingly, the work of Gluais P et al ~10 years later found that the amount of PGI₂ evoked by ACh was much higher (~10-100-fold) than those of other prostanoids and that the contraction to ACh, which was present in SHR but not in control WKY aortas, was abolished by TP antagonism. Furthermore, they found that the application of PGI₂ itself caused an increased contraction in SHRs as against that of control WKYs and such a response was again inhibited by TP antagonism. In this study and subsequent ones of these authors, IP receptor was found to be dysfunctional in the diseased vessels or upon aging. It was thus proposed that in diseases or upon aging, the dysfunction of IP together with an excess of production would result in PGI₂ (which normally evokes relaxation in health) overflowing to act on TP, making it function mainly as an EDCF under pathological conditions. This does explain to a certain extent why endothelium-dependent contractions were originally readily detectable in SHRs and diabetic or aging rats, but hardly seen in normal and young counterparts.

2.2 | PGI₂’s acting as an EDCF in normal vessels and its ability to naturally activate TP along with IP

Endothelium-dependent contraction also frequently occurs in normal vessels. Moreover, we have found that in many such vessels PGI₂ is also detected as the predominant COX product and an extraneous application of the compound evokes robust contractions sensitive to TP antagonism. Interestingly, in some vessels, e.g., mesenteric arteries that even show dilatation to PGI₂, TP antagonism enhances the relaxing response (Figure 1A). Meanwhile, in those, such as mouse carotid arteries that show a contractile response, the contraction evoked by PGI₂ is increased by IP antagonism (Figure 1B). A prior report also showed that antagonizing TP causes a dilator response to PGI₂ in rat aortas. These findings together not only suggest that TP can be activated by PGI₂ independent of vessel types, but also imply that it can be naturally activated along with IP, reflecting an intrinsic property of the receptor to be activated by PGI₂.

Since IP and TP are all widely expressed among vascular beds, we have proposed that the opposite responses to PGI₂ previously seen among vascular beds can be the resultants

![Figure 1](attachment:image_url)

**Figure 1** Enhancements of Tx-prostanoid receptor (TP) or I prostanoid receptor (IP) antagonism on the divergent prostaglandin I₂ (PGI₂)-evoked responses. (A) TP antagonism with SQ29548 (10 μM) increases the relaxation to PGI₂ in NOS-inhibited, phenylephrine (2 μM) precontracted mouse mesenteric arteries. (B) IP antagonism with CAY10441 (1 μM) increases the contraction to PGI₂ in NOS inhibited mouse carotid arteries. MA, mesenteric arteries; CA, carotid arteries. **p < .01 versus controls. Modified from Liu et al.**
from vasoconstrictor activities outweighing or being outweighed by the concurrent dilator activities. In agreeing with the idea, the response of the COX substrate AA in mouse mesenteric arteries is also oppositely influenced by TP or IP antagonism, implying that the in situ natively produced PGI$_2$ has similar effects. Moreover, in mice with TP deficiency (TP$^-/-$), we have not only confirmed the involvement of TP in the PGI$_2$- or EDCF-evoked responses, but also suggested that TP can be a common vasoconstrictor receptor of all other COX-derived prostanoids, which per se implies that the activation of the receptor by PGI$_2$ results from the structural similarities inherited among prostanoids. In fact, this may also explain why another prostanoid receptor, the F prostanoid receptor (the prototype receptor of PGF$_2\alpha$) is activated by non-F prostanoids, such as PGD$_2$, and other unexpected effects shown by many prostanoids or receptor antagonists.

### 2.3 Involvement of EP3 in PGI$_2$’s EDCF-like action

In some vessels the contractile response of PGI$_2$ or EDCF is less sensitive to TP antagonism that completely abolishes the response evoked by the most potent TP agonist, that is, the TxA$_2$ analogue U46619. Also, in some of such vessels IP is found to be expressed; however, PGI$_2$ is unable to cause relaxation after TP antagonism. These results imply the presence of additional contractile receptor(s) involved in PGI$_2$’s or EDCF’s contractile activities. Indeed, the involvement of non-TP receptor(s) in EDCF activity has also been suggested previously; however, the exact underlying mechanism or identity of the receptor(s) has not been elucidated.

To address the issue, we have performed experiments on TP$^-/-$ mouse vessels. Our results showed that in the endothelium-denuded (Figure 2A) or NO synthase (NOS) inhibited (with Nω-nitro-L-arginine methyl ester; L-NAME) TP$^-/-$ abdominal aorta, PGI$_2$ still evokes a contractile activity under precontracted conditions (under which a contractile activity can be more readily detected), although its response under the baseline conditions is largely removed. Interestingly, a relaxation is observed in such vessels when EP3 is antagonized (Figure 2A). Similar pattern of responses is also obtained with TP-inhibited WT and EP3$^-/-$ counterparts under NOS inhibited conditions (Figure 2B). These results indicate that PGI$_2$ also activates EP3 along with IP; hence, the contraction observed reflects the contractile activities of the prostanoid resulting from both TP and EP3 concurrently activated. It should be noted that the removal of EP3-mediated effects has a similar effect on ACh-evoked responses, verifying the vasoconstrictor property of EDCF being similar to that of the extraneously applied PGI$_2$.

### 2.4 Properties of EP3 in activation by PGI$_2$ related to those of IP or TP

Although PGI$_2$ is known to evoke contractile activity at high concentrations in many vascular beds, a contraction rather than a relaxation evoked by the prostanoid at very low concentrations is seen in vessels that has a substantial expression of EP3. A possibility thus exists that the action of PGI$_2$ on EP3 could be more potent than that on TP. To substantiate this, we have studied the effects of TP$^-/-$ and/or EP3$^-/-$ on PGI$_2$ responses in mouse renal arteries where not only is the
expression of IP detected, but TP and EP3 are also found to be expressed in a higher level than in many other vessels.\textsuperscript{73,75,76} As shown in Figure 3A, under baseline conditions EP3$^{-/-}$ does reduce the maximal contraction to PGI$_2$ (10 μM), but to a lesser extent than TP$^{-/-}$. Interestingly, EP3$^{-/-}$ but not TP$^{-/-}$ abolishes the contraction to the prostanoid at 0.1 μM, although it has a much lesser effect than TP$^{-/-}$ on the maximal response as stated above. This may suggest that the potency of PGI$_2$ on EP3 is higher but its efficacy on EP3 is lower than that on TP.

At the same time, we noted that in such treated vessels PGI$_2$ still evokes contraction from 0.01 μM in TP$^{-/-}$ renal arteries under precontracted conditions (although smaller than that of WT controls where contraction can be seen from 0.001 μM), but it causes relaxation in EP3$^{-/-}$ counterparts (Figure 3B). In addition, TP$^{-/-}$/EP3$^{-/-}$ can only significantly increase the relaxation caused by PGI$_2$ at 0.1 μM or higher (Figure 3B). These results not only verify a higher potency of PGI$_2$ on EP3 than on TP ($\sim$1 log behind; estimated by comparing the initial effects shown by the deficiency of one receptor), but also further suggest that the potency of PGI$_2$ on EP3 is higher than or at least similar to that on IP (estimated by the contractile PGI$_2$ response even after TP$^{-/-}$ as well as the initial effect of EP3$^{-/-}$ alone or that with TP$^{-/-}$ on PGI$_2$-responses under precontracted conditions). Because PGI$_2$ does not evoke relaxation in the TP$^{-/-}$ vessels, the efficacy of the prostanoid on EP3 can be higher than that on IP as well (only in vessels with abundant EP3). Moreover, the differences in effects between TP$^{-/-}$ and EP3$^{-/-}$ on PGI$_2$ responses in renal and other vessels, such as abdominal aortas,\textsuperscript{78} suggest that the action of PGI$_2$ on TP alone starts from $\sim$0.1 μM, and its effect at $\leq$1 μM is still smaller than that of EP3 and can be easily overcome by the concurrent dilator effect of IP, on which PGI$_2$ has a higher potency as stated above.

It should be noted that our results have shown that the deletion of one of TP and EP3 does not alter the expression or function of the other. In addition, the expression of IP is not altered by TP$^{-/-}$ and/or EP3$^{-/-}$ \textsuperscript{49,73,79} These results rule out that the above-mentioned differential effects of TP$^{-/-}$ and EP3$^{-/-}$ on PGI$_2$ responses result from altered expressions or functions of the remaining receptor(s) of these two and/or that of IP. In addition, the involvements of TP and/or EP3 in PGI$_2$ contractile activities do not limit to large arteries. For example, in perfused mouse kidneys, PGI$_2$, which normally does not evoke any relaxation, causes a contraction from 1 μM in WT mice, yet a relaxation in EP3$^{-/-}$ counterparts that further increases when TP is also absent (Figure 4A–C). Similar responses may be obtained with its analogue iloprost (Figure 4D). A contraction to PGI$_2$ was also observed in porcine intra-renal arteries, although the role of EP3 was not studied in the report.\textsuperscript{50} Besides, we have shown that TP- and EP3-mediated vasoconstrictor activities drastically decrease the dilator action of PGI$_2$ in rat mesenteric resistance arteries.\textsuperscript{79} In fact, there is one report showing that low dosages of PGI$_2$ that were unable to evoke a systemic depressor response caused renal vascular constriction in vivo.\textsuperscript{80}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{TP$^{-/-}$ and EP3$^{-/-}$ differentially influence prostaglandin I$_2$ (PGI$_2$)-evoked contractile responses in NOS-inhibited mouse renal arteries. (A) Response of PGI$_2$ (0.01-10 μM) in WT, TP$^{-/-}$, EP3$^{-/-}$ or TP$^{-/-}$/EP3$^{-/-}$ vessels obtained under baseline conditions. (B) Response of PGI$_2$ (0.001-0.1 μM) in WT, TP$^{-/-}$, EP3$^{-/-}$ or TP$^{-/-}$/EP3$^{-/-}$ vessels precontracted with phenylephrine (1 μM). *p < .05; **p < .01 versus WT; +p < .05; ++p < .01 versus TP$^{-/-}$; ***p < .01 versus EP3$^{-/-}$. Modified from Liu et al\textsuperscript{78}}
\end{figure}
3 | VARIED RESPONSES EVOKED BY PGI₂ AMONG VESSELS AND RELATION OF ITS DILATOR ACTIVITY TO EDHF

3.1 | Varied responses evoked by PGI₂

As discussed above, the actions of PGI₂ on TP, EP3, and IP vary in potency as well as in efficacy. Also, the expressions or functions of these three receptors differ among vascular beds. Compared to those of other two receptors, the expression of EP3 is limited to smaller number of vessels. Also, although the expressions of TP and IP are found in most vessels, variations in the expression level or function, especially that of IP also exist. In accordance, different patterns of responses evoked by PGI₂ at different concentrations or in different vascular beds would be expected even under normal physiological conditions (Figure 5).

In vessels with little expression or function of EP3 (e.g., mouse mesenteric arteries), the response to PGI₂ is mainly determined by those of IP and TP. Therefore, in such vessels a relaxation is commonly seen at low concentrations (≤1 μM), but a contraction or a relaxation compromised by...
a vasoconstrictor activity at higher ones (≥1 μM), due to a higher potency of the prostanoid on IP than on TP and/or the difference in the relative expression levels or functions of these two receptors (Figure 5). Indeed, initial relaxations to low concentrations of PGI2 followed by contractile responses evoked by higher ones of the compound have been frequently observed across species. On the other hand, when such vessels, for example, mouse carotid arteries, have a low expression level of IP, the response to PGI2 could also possibly be only a contraction that starts from 1 μM or higher.

Meanwhile, in those with abundant EP3 expression or function (e.g., mouse renal arteries and abdominal aorta), a contraction is not only observed to start from a very low concentration (0.001 or 0.003 μM) due to the action of EP3 alone being able to outweigh that of IP, but also seen with a robust maximal response reflecting activities of both TP and EP3 outweighing that of IP (Figure 5). Also, because of the high potency of EP3-mediated effect, the dilator activity of PGI2 of low concentrations (≤1 μM) in some vessels even with a relatively lower expression or less function of the receptor as against that of IP could be substantially compromised by a vasoconstrictor action derived from EP3 (Figure 5). For example, PGI2 (at 1 μM) evokes only a minor relaxation in WKY mesenteric arteries, which is however increased to a complete relaxation by antagonizing both EP3 and TP, but is only slightly increased by that of TP alone.

Also, the expressions of TP, EP3, and IP or production of PGI2 can be altered under disease conditions (Figure 5). For example, the expressions or functions of TP and/or EP3 have been reported to increase while that of IP may decrease in disease conditions, such as hypertension and atherosclerosis. Moreover, the production of endothelial PGI2 may also increase (Figure 5). These factors may tip the action of PGI2 toward mediating vasoconstrictor actions or implicating in endothelial dysfunction.

3.2 Relation of the dilator effect of PGI2 to EDHF

In addition to PGI2, the endothelium also releases other NO-independent vasoactive substance(s), for example,
endothelium-dependent hyperpolarizing factor (EDHF),
which mediates vasodilator actions. NO-independent
derived vasodilator mechanisms play important roles in
vascular diseases when endothelial NO availability is impaired. How the vasodilator activity of
PGI₂ via IP, which is also NO-independent, interacts with
EDHF could be elucidated by comparing results from
NOS-inhibited mouse abdominal aortas after COX inhibition
and those following TP and EP₃ antagonisms. In
NOS-inhibited such vessels ACh, which is commonly seen to evoke contraction, results in a robust relaxation after antagonizing both TP and EP₃ but not after antagonizing TP alone. Such a relaxation is, however, significantly reduced when COX is also inhibited (Figure 6A). These results suggest the existence of dilator activities mediated by both EDHF and IP.

Interestingly, in those with atherosclerosis, while the NO-independent relaxation to ACh after COX inhibition (which reflects EDHF activity) is significantly reduced, that after TP and EP₃ antagonisms (which reflects

![Figure 6](image-url)

**FIGURE 6** Relation of prostaglandin I₂ (PGI₂)-mediated dilation to that of endothelium-dependent hyperpolarizing factor. (A) Time-course of the response to acetylcholine (Ach; 10 μM) in NO synthase-inhibited, phenylephrine (2 μM) precontracted WT mouse abdominal aorta with Tx-prostanoid receptor (TP) antagonized (SQ29548; 10 μM), that additionally with E prostanoid receptors 3 (EP₃) antagonized (L798106; 1 μM), or that additionally with EP₃ antagonized and cyclooxygenase (COX) inhibited (indomethacin; 10 μM). **p < .01 TP antagonism versus TP antagonism/TP and EP₃ antagonists; ††p < .01 TP and EP₃ antagonisms versus TP and EP₃ antagonisms/COX inhibition. (B) The maximal response to Ach (10 μM) in NOS-inhibited non-atherosclerotic abdominal aortic rings of WT mice (WT) with COX inhibited (with indomethacin; Ach/COX inhibition) or TP and EP₃ antagonized (with SQ29548 and L798106; Ach/TP and EP₃ antagonisms) and that of rings with atherosclerotic lesions from ApoE⁻/⁻ mice fed on fat (ApoE⁻/⁻). **p < .01 versus Ach/COX inhibition; ++p < .01 versus Non-AS/WT. Modified from Li et al and Hu et al.
a resultant from both EDHF and IP-mediated effects) is comparable to the response of non-atherosclerotic control vessels (Figure 6B). This may imply that the dilator activity of IP does not have an additive effect with that of EDHF, and the resulted response derived from the presence of both IP and EDHF probably only reflects the larger one of the two similar effects, due to a redundancy of NO-independent dilator mechanisms suggested previously.Indeed, antagonizing IP increases the contraction evoked by PGI₂ itself (a response reflecting contractile activities overcoming a dilator effect from IP) but not that of ACh (a response reflecting contractile activities overcoming the dilator effects from both IP and EDHF) under NOS inhibited conditions.

The above idea also explains that in NOS-inhibited mouse renal arteries where both PGI₂-mediated dilator activity and that of EDHF can be detected, TP−/−/EP3−/− causes a relaxation to ACh of an extent similar to that obtained with COX inhibition. Also, in mouse mesenteric arteries where COX-1-derived PGI₂ alone can be observed to evoke relaxation, a relaxing activity derived from PGI₂ resulting from endothelial stimulation (which stimulates the production of PGI₂ and EDHF) appears rather elusive, which could also be possibly due to the redundancy resulting from the existence of a robust EDHF-mediated dilator activity.

4 | VASCULAR PATHOLOGIES AND THERAPEUTIC IMPLICATIONS

4.1 | Hypertension

Hypertension is commonly thought to be associated with endothelial dysfunction, which could result from reduced endothelial NO bioavailability and/or increased EDCF activity. Indeed, many vascular beds of hypertensive conditions show increased EDCF activity due to an increase in PGI₂ production or in expressions of TP and EP3, which can be associated with or without the presence of IP dysfunction. In addition, the increased TP and/or EP3 expressions of hypertensive conditions could result either from up-regulation of their expressions or from a reduced down-regulation of these receptors during the transition from adolescence to adulthood.

Notably, PGI₂ may directly constrict some intra-renal arteries or the whole renal vasculature (which takes an important part in regulating systemic blood pressure) via the effects of TP and/or EP3 outweighing that of IP. This further underscores the pathological role of the increased EDCF activity under the disease conditions. Indeed, inactivating TP and/or EP3 as well as COX-1 inhibition or COX-1−/− (which also removes endothelial PGI₂ synthesis) has been shown to alleviate the development of angiotensin II (Ang II) or Ang II-salt induced hypertension, diabetic or renal hypertension as well as hypertension in SHRs, although such an effect could also be related to non-PGI₂ mediated effects.

4.2 | Atherosclerosis

Endothelial dysfunction resulting from EDCF-mediated action is also commonly associated with atherosclerotic vessels and it may play an important role in the development of ischemic events. In the lesion area, not only is NO bioavailability reduced, but EDHF is also impaired. Accordingly, endothelial PGI₂ synthesis has an increased role in regulating vessel function of atherosclerotic conditions. Interestingly, the production of PGI₂ may increase in atherosclerosis. Moreover, we have previously shown that in the lesion area of atherosclerotic mouse aortas where PGI₂ evokes contraction ACh, which commonly elicits relaxation with NOS uninhibited, evokes a contractile response under the same NOS uninhibited conditions. Importantly, this contractile response is reversed by the removal of both TP- and EP3-mediated effects but not by that of TP alone into a relaxation that adds to the reduced endothelium-mediated dilator mechanism resulting from impaired NO and EDHF activity.

The above results suggest that maximally uncovering the dilator activity of natively produced PGI₂ with TP and EP3 antagonisms can effectively relieve endothelial dysfunction under the atherosclerotic or other pathological conditions. It needs to be pointed out that PGI₂ may evoke contraction in coronary circulation. A contraction to ACh can be observed in atherosclerotic human coronary arteries. COX inhibition has also been found to improve ACh-evoked relaxation in atherosclerotic patients. However, further studies are still needed to elucidate the precise role of PGI₂ in coronary heart diseases and its association with TP and EP3 activations.

4.3 | Diabetes

Diabetes is also commonly suggested to be associated with endothelial dysfunction with increased EDCF activity, although various mechanisms have been proposed. It must be emphasized that under diabetic conditions, PGI₂ may still remain as the predominant endothelial COX product. In addition, there are studies showing that in diabetic coronary arteries, the response to PGI₂ can be reversed from dilatation to contraction. Consistent with these results, COX-1−/− (which
removes endothelial PGI₂ synthesis under diabetic conditions, TP⁻/⁻, or EP3⁻/⁻ has been reported to improve diabetic complications, such as nephropathy.⁵¹,¹¹⁰,¹¹¹ although the causal relationship of such an effect with the improvement of endothelial dysfunction remains to be established.

4.4 | Therapeutic implications

As we discussed above, the fundamental vasoregulating mechanisms of PGI₂ may differ considerably from the originally proposed; hence, there should also be significant considerations in therapeutic management of cardiovascular disorders. Since the PGI₁ signal pathway through IP and that via TP and EP3 play opposing roles in endothelial dysfunction of disease conditions, antagonizing both TP and EP3 can thus be proposed to maximally uncover the beneficial effect of PGI₂ via IP.⁸⁶,⁸⁹ Such an approach could be advantageous over the current one using low dosage of aspirin (which is suggested to selectively inhibit COX in platelets) in ischemic heart diseases.¹² In fact, endothelium-specific or global COX-1⁻/⁻, global TP⁻/⁻ as well as COX-1 or TP inhibition but not that in platelets has been found to improve endothelial function or alleviate the development of atherosclerosis,⁴⁸,⁵²,⁸⁹,¹⁰³,¹¹²–¹¹⁵ underscoring the important pathological roles of TP and EP3 activations derived from the endothelial COX catalyzed metabolism that mainly produces PGI₂.

The above results from PGI₂’s action also explain why many PGI₂ analogues, whose designs are derived from PGI₂ structurally, have been previously found to activate EP3 and TP as well.²,⁵⁰,¹¹⁶,¹¹⁷ It should be noted that these analogues and PGI₂ itself are clinically used for pulmonary hypertension and peripheral vascular diseases¹¹⁸,¹¹⁹; hence, their adverse effects via TP and EP3 on certain vascular beds, especially the renal vasculature,⁷⁸ can be no longer ignored.

5 | QUESTIONS REMAINING TO BE RESOLVED OR LIMITATIONS OF RESULTS OBTAINED FROM GENETICALLY ALTERED MICE

5.1 | Precise properties of PGI₂’s action on TP and EP3 and its precise role in endothelium-dependent contractions

Although the potency and efficacy of PGI₂ on TP or EP3 relative to those on IP can be roughly estimated by the effects of TP⁻/⁻ and/or EP3⁻/⁻ to explain the varied responses evoked by the prostanoid across vascular beds as described above, their precise values still need to be explicitly elucidated by experiments using tissues or cells expressing only one type of these prostanoid receptors. These values are of special interest evaluating the precise role of PGI₂ in endothelium-dependent contraction of vessels where other prostanoids, such as PGE₂ and PGF₂α, and some non-COX AA metabolites for example, isoprostanes that also activate TP and/or EP3, could also be released although to a lesser extent.⁴⁷,⁷⁸,¹¹²–¹¹⁵ In fact, in some vascular beds PGF₂α has been found to be the major EDCF, although it is produced in a lesser amount than PGI₂, due to the prostanoïd’s evoking vasoconstriction at a very low concentration here where PGI₂ does not show any contractile effect.³³–³⁶ On the other hand, such an inconsistency as against our reports could result from variations among species or vascular beds. For example, in many vessels, PGF₂α is found to evoke contractions similar to PGI₂⁴,⁷³,⁷⁵,⁷⁸ or its production is hardly detectable while PGI₂ is abundantly produced.⁹,⁵⁰,⁷⁹

5.2 | The applicability of PGI₂’s functioning as an EDCF in humans

COX inhibition has been reported to remove EDCF activity that also takes part in the regulation of human vascular function, especially under disease conditions.⁵⁴,⁹⁹,¹⁰¹,¹²⁴–¹²⁷ PGI₂ has been reported to evoke vasoconstrictor effect or to have limited vasodilator effect in some vascular beds of humans.⁶³,⁶⁴,¹²⁸ It should be noted that TP and EP3 are all reported to function and mediate contractions in human vessels.²,⁴,¹²⁹–¹³¹ Moreover, TP has been reported to mediate PGI₁’s vasoconstrictor effect in human renal arteries.⁸³ On the other hand, there has yet been no direct evidence to verify that PGI₂ functions as an EDCF in humans. Also, it remains to be determined whether the weak vasoconstrictor effect of PGI₁ reported in some humans vessels is due to limited vessels studied or the scarce vasodilator effect of low concentrations of PGI₂ shown on some human vascular beds results from the compromising effect of EP3 as we have noted in rat mesenteric resistance arteries.⁵³,⁷⁹,¹²⁸ It needs to be pointed out that although the vasoconstrictor effect of PGI₂ in some vascular beds might be subtle under normal conditions it can significantly increase in diseases.⁷⁹,⁸⁶

5.3 | The applicability of PGI₂’s effect on TP and EP3 in platelets

TP and EP3 are also expressed in other tissues or cell types, including platelets.² Interestingly, endothelial PGI₂ seems to have a definite anti-platelets-aggregating effect.¹³²
which appears to contradict with the results we found on vessels. However, in this circumstance, considerations should also be given to the influences of other prostanoids (e.g., TxA₂ and PGE₂) that are produced in platelets to activate TP and EP₃. The expression level of IP as well as the amount of PGI₂ diffused to platelets and the diluting effect of the blood stream. Again, the precise potency and efficacy of PGI₂ on TP or EP₃ related to those on IP or to those of other prostanoids produced in platelets are needed to elucidate the precise interactions of TP and EP₃ with IP in PGI₂-stimulated platelets.

6 CONCLUSION

Although commonly recognized as a vasodilator through IP, PGI₂ may also function as a major EDCF and its responses vary at different concentrations, among different vascular beds, or between physiological and pathological conditions. This is due to the prostanoid activating TP, EP₃, and IP with differences in potency and/or efficacy and the varied expressions or functions of these receptors that can alter during pathological conditions (Figure 5). Such an idea differs considerably from the classical concept of the endothelial COX pathway in regulating vascular function. Therefore, considerations should be taken regarding the adverse effects of TP and EP₃ during the current clinical practices of using PGI₂ or its analogues that activate TP and EP₃ or low dosage of aspirin that leaves the endothelial COX pathway untouched in patients with cardiovascular disorders. However, studies are still needed to explicitly establish the precise properties of PGI₂’s action on TP and EP₃ relative to those on IP or to those of other prostanoids and the vasoconstrictor role of PGI₂ through TP and EP₃ in humans, especially in those with coronary heart diseases. Also, how PGI₂’s action on TP and EP₃ interacts with that on IP in platelets might also require further investigation.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (31771272 and 81970433 to YZ and 81770678 and 82070506 to BL), and by the Natural Science Foundation of Guangdong Province (2019A1515011650 to BL).

DISCLOSURES

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Bin Liu and Yingbi Zhou contributed to the original writing and editing of the manuscript. Yingbi Zhou composed the figures for the manuscript.

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How to cite this article: Liu B, Zhou Y. Endothelium-dependent contraction: The non-classical action of endothelial prostacyclin, its underlying mechanisms, and implications. *FASEB J.* 2021;35:e21877. [https://doi.org/10.1096/fj.202101077R](https://doi.org/10.1096/fj.202101077R)