Sympathy for the devil: the role of thromboxane in the regulation of vascular tone and blood pressure

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Sellers MM, Stallone JN. Sympathy for the devil: the role of thromboxane in the regulation of vascular tone and blood pressure. Am J Physiol Heart Circ Physiol 294: H1978–H1986, 2008.—Historically, the vasodilatory prostanoids, especially prostacyclin and prostaglandin E2, are believed to contribute significantly to the regulation of normal vascular tone and blood pressure (BP), primarily by counteracting the prevailing effects of the systemic vasoconstrictor systems, including angiotensin II, the catecholamines, and vasopressin. In contrast, the primary vasoconstrictor prostanoid thromboxane A2 (TxA2) is produced in far smaller quantities in the normal state. While TxA2 is believed to play a significant role in a variety of cardiovascular diseases, such as myocardial infarction, cerebral vasospasm, hypertension, preeclampsia, and various thrombotic disorders, its role in the regulation of vascular tone and BP in the normal physiological state is, at best, uncertain. Numerous studies have firmly established the dogma that TxA2, while important in pathophysiological states in males, plays little or no role in the regulation of vascular tone or BP in females, except in the pulmonary vasculature. However, this concept is largely based on the predominant and preferential use of males in animal and human studies. Recent studies from our laboratory and others challenge this dogma and reveal that the TxA2 pathway in the systemic vascular beds, the vasodilatory prostanoids in the regulation of vascular function, present more recent findings that challenge this dogma, and, finally, provide a new perspective on the role of the constrictor prostanoids in the regulation of vascular function and BP.

Biosynthesis, Signal Transduction Mechanisms, and Actions of TxA2

Biosynthesis. Biosynthesis of TxA2 and the PG involves the sequential metabolism of AA in three stages. The first rate-limiting step is the stimulus-induced mobilization of AA from

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THE PROSTANOIDS ARE THE MAJOR class of vasoactive eicosanoids (oxygenated 20-carbon fatty acids) derived from the metabolism of arachidonic acid (AA) by the endothelium (Endo) and/or vascular smooth muscle (VSM) of the blood vessel wall and include the prostaglandins (PG), PGD2, PGE2, PGF2α, and PGI2 (prostacyclin), and the thromboxanes (TxA2; Ref. 71). It is well established that these vasoactive substances play important roles in the local regulation of vascular tone, in both normal physiological and pathophysiological states. In most systemic vascular beds, the vasodilatory prostanoids prostacyclin and PGE2 are the major products of the vascular wall. There is considerable evidence that these substances, together with nitric oxide (NO), play an important role in the regulation of normal vascular tone and blood pressure (BP), either by mediating the actions of vasodilator substances or by modulating the actions of systemic vasoconstrictor hormones, such as angiotensin II (ANG II), the catecholamines, and vasopres-
cell membrane phosphoglycerides by the enzymes phospholipase (PL) A2 (acting on phosphatidylcholine) and diacylglycerol (DAG) lipase (acting on phosphatidylinositol). The second is the oxidation of AA to the PG endoperoxides by the enzyme PG endoperoxide synthase, also known as cyclooxygenase (COX). This involves two sequential actions by the enzyme: first, the conversion of AA to endoperoxide PGG2 (COX activity), and, subsequently, the conversion of PGG2 to endoperoxide PGH2 (peroxidase activity). In the third and final stage, PGH2 is isomerized into the biologically active prostanoïd end products by specific PG synthase enzymes (9, 59, 71). For TxA2 biosynthesis, this involves the action of TxA2 synthase (TxS) on PGH2 to produce TxA2. The profile of prostanoïd end products produced within each tissue varies and is determined primarily by the amounts of the specific PG synthase enzymes present. In most vascular beds, prostacyclin is the most abundant end product of AA metabolism by the Endo and/or VSM cells. Well-established examples involve the effects of ANG II and VP, which act directly on VSM receptors to induce vasoconstriction but also interact with their Endo and VSM receptors to activate the release of AA and biosynthesis of prostacyclin and PGE2, which then act locally to modulate the vasoconstrictor actions of these systemic hormones on vascular tone and BP (17, 53, 57). Similarly, humoral activation of the platelets by ADP, thrombin, and TXA2 itself stimulates the release of AA and prostanoïd biosynthesis, principally TXA2 (54).

Cell membrane-bound AA also may be metabolized to form a group of compounds known as the isoprostanes. These relatively recently identified compounds are autoxidation products of AA catalyzed by free radicals instead of the COX enzymes. The isoprostanes are chemically and biologically similar to the enzymatically produced prostanoïds but must be released from the cell membrane by PLs to exert their biological effects. Isoprostane synthesis begins with free radical attack of membrane-bound AA to form arachidonoyl radicals, which then undergo sequential peroxidation to form H2-isoprostanoid endoperoxides and then partial or complete reduction to form four classes of esterified isoprostanes similar to the prostanoïds (i.e., D2, E2, F2, and isothromboxanes). Deesterification by PLs then releases the isoprostanes, allowing the free molecules to exert their biological actions (23, 24, 55). Because isoprostane formation is primarily dependent on free radical activity, their production may be used as a marker for levels of oxidative stress and lipid peroxidation in human CV diseases. Thus enhanced tissue levels of isoprostanes detected in various diseases suggest that free radicals play a central role in the pathogenesis of CV diseases, such as atherosclerosis, inflammation, and ischemia-reperfusion injury (24, 55, 56).

All four classes of isoprostanes exert TXA2-like biological effects on both animal and human blood vessels, which include vasoconstriction and proliferation (mitogenesis) in VSM and aggregation of platelets, through interaction with prostanoïd receptors. The F2-isoprostanes exert potent effects on the vasculature, and TXA2 receptor antagonist experiments provide convincing evidence that the TXA2 receptor mediates all of the observed vascular effects in vivo and in vitro, with the F2-isoprostanes acting as full or partial agonists (24, 55). Similar to TXA2, the vascular effects of the isoprostanes are linked via the TXA2 receptor to Ca2+ influx, the formation of inositol trisphosphate (IP3) and DAG, and the activation of protein kinase C (PKC). While the E2-isoprostanes exert more potent vascular effects than the F2-isoprostanes, data regarding the vascular actions of the A2-isothromboxanes are so far unavailable due to the instability of these compounds. The available data strongly suggest that the F2-isoprostanes (and perhaps other isoprostanes) are involved in the pathogenesis of CV diseases involving oxidative stress, and these compounds may act synergistically with COX-derived TXA2 through the TXA2 receptor to induce pathophysiological effects on the vasculature. Clearly, more studies are required before the interactions between these parallel products of AA metabolism are fully understood.

Signal transduction pathways. The acute and chronic effects of TXA2 are mediated by the TXA2 (TP) receptor, a member of the prostanoïd family of eight transmembrane-spanning heterotrimeric G protein-coupled receptors, which share ~20–30% sequence homology with each other (9, 12). The human TP receptor was the first eicosanoid receptor to be cloned and exists as two alternatively spliced variants, TP-α (from placenta and platelet) and TP-β (from Endo) that differ in the length and sequence of the carboxyl tail. Interestingly, the mRNAs for both splice variants have been identified in many tissues that express the TP receptor, including brain, platelets, placenta, VSM, small intestine, and thymus (9, 37). Vascular
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Endo cells may also express both variants, but the available evidence is contradictory at present (2, 37). No differences have been observed in ligand binding or coupling of TP-α and TP-β receptor splice variants, and both are rapidly phosphorylated and desensitized following agonist binding. However, following exposure to agonist, TP-β appears to internalize to a much greater extent than TP-α (9, 37). Interestingly, TP-α also undergoes heterologous desensitization in response to prostacyclin and NO stimulation, which involves PKA- and PKC-mediated phosphorylation of serine residues in the novel TP-α COOH-terminal tail (37, 84). There is also evidence of reciprocal heterologous regulation of the TP and prostacyclin (IP) receptors through heterodimerization of IP and TP, which modifies the cellular responses to both prostanoids (84, 87). These findings raise the intriguing possibility that the effects of TxA2-PGI2 balance on vascular homeostasis may involve differences in TP and IP receptor function as well as differences in the tissue levels of these prostanoids. The role of estrogen in regulating these IP-TP receptor interactions in Endo and VSM is unclear at present, and future studies could provide important new information on the mechanisms by which the constrictor prostanoids regulate vascular function and structure.

There is abundant evidence that the TP receptor is coupled to the G4-type G protein in VSM and platelets, which mediates activation of PLC (PLC-β). This membrane-bound enzyme hydrolyzes phosphatidylinositol to form the second messengers IP3 and DAG (9, 37). IP3 activates the mobilization of intracellular Ca2+, whereas DAG activates PKC. TP-mediated activation of PLC-β and the subsequent release of intracellular Ca2+ are the primary events in TxA2/TP receptor signaling and, via PKC, lead to the induction of platelet aggregation and VSM contraction. PKC activation may also mediate the chronic effects of TxA2, such as VSM hypertrophy (9, 37, 41). TxA2-induced signaling via the TP receptor involves activation of p38 MAPK, which is essential for platelet aggregation at low concentrations of TxA2; in contrast, activation of PKC is critical for TxA2-induced platelet aggregation and VSM contraction and hypertrophy. Finally, TxA2 activation of MAPK, together with various growth factors, could lead to mitogenesis in VSM and other cell types (9). Functional evidence of the importance of this signaling pathway is revealed by the linkage between defects in TP receptor signaling with disruption of IP3 production and coagulation deficiencies (37).

The presence of the TP receptor in the vascular Endo has been established by a variety of biochemical, functional, and molecular evidence, including TxA2 receptor binding (36, 76), TxA2-induced release of endothelium-dependent vasodilatory substances (28, 39), and cloning of the TP receptor (2, 37). The TP-β splice variant was first identified in the vascular Endo (9, 37). Although it is unclear whether Endo cells express both isoforms of the TP receptor, no differences have been observed in ligand binding or coupling of TP-α versus TP-β. TP receptor(s) in the Endo (as in other cell types) are coupled to Gαi, which mediates an increase in intracellular Ca2+ and activation of PKC (1, 41). The TP-β receptor is also coupled to Gαi2, which modulates Endo cyclic AMP content by its inhibitory action on adenyl cyclase (1, 32).

Acute and chronic actions on the vascular wall. TxA2 exerts both acute and chronic effects on the vascular wall. Historically, the acute effects of TxA2 have been related to its role as a prothrombotic agent; thus it is both a potent vasoconstrictor and a powerful activator of platelet aggregation and shape change, effects of primary importance in the initial activation of hemostatic mechanisms (17, 35, 54). With the tremendous explosion of research over the last 30 years focused on elucidating the roles of prostanoids in CV health and disease, there has been increasing appreciation that the powerful constrictor action of TxA2 on VSM (greater than that of ANG II) may also play an important role in the regulation of local vascular tone, at least in pathophysiological states such as HT and heart failure. More recent studies have also highlighted the chronic effect of TxA2 to promote hypertrophy of VSM and abnormal remodeling of the vascular wall (9, 12, 13, 35). The extent of these acute and chronic effects of TxA2 on clotting mechanisms and the vascular wall appear to depend, at least in part, on the relative balance of its effects with those of the vasodilatory prostanoids (primarily prostacyclin), which also exert anti-aggregatory effects on platelets and inhibit VSM hypertrophy (17, 21, 54, 79). Of particular functional significance to the regulation of constrictor prostanoid function is the equal ability of both TxA2 and its endoperoxide precursor PGG2 to bind to the TP receptor and exert biological effects on the platelet and vascular wall (35, 41, 50). Indeed, PGG2 accumulates when TxS is inhibited and continues to activate the TP receptor in platelets and VSM in the absence of TxA2 (50). Numerous studies utilizing TxS inhibitors, such as dazoxiben and CGS-13080, and the TP receptor antagonist SQ-29548 (SQ) have demonstrated that PGG2 as well as TxA2 contributes to the elevated arterial vasoconstriction or BP in various rat models of HT (3, 16, 33, 47, 48, 64). Similarly, in pathophysiological conditions where there is significant oxidative stress, such as ANG II-induced HT, free radicals may activate the VSM TP receptor independent of PGG2 or TxA2.

The biochemical and molecular evidence of TP receptors in the Endo suggests that the effects of TxA2 on intact blood vessels are more complex than its effects on VSM alone. The functional evidence that constrictor prostanoids exert effects on the Endo and/or vascular function reveals that this indeed is true. Thus both platelet-derived TxA2 and TxA2 mimetics induce the release of endothelium-derived vasodilator substances from the intact arterial wall (28, 39). Similarly, in cultured human venous endothelial cells, the TxA2 mimetic U-46619 produces concentration-dependent increases in intracellular Ca2+ and the release of prostacyclin (41). These findings strongly suggest that the acute and chronic effects of TxA2 on the vascular wall depend on the physiological or pathophysiological state of Endo as well as VSM function.

The Role of TxA2 in CV Homeostasis: Historical Viewpoint

Role of TxA2 in normal CV homeostasis. Historically, the vasodilatory prostanoids, especially prostacyclin and PGE2, are believed to contribute significantly to the regulation of normal vascular tone and BP, primarily by counteracting the prevailing effects of the systemic vasoconstrictor systems, including ANG II, the catecholamines, and VP. Indeed, numerous studies have confirmed that inhibition of prostanoid synthesis increases vascular tone in vitro and BP in vivo (17, 53, 69). In contrast, much smaller amounts of TxA2 are produced by the vascular wall, and inhibition of TxS or blockade of the TP receptor in normotensive (NT) animals and humans does not alter BP; thus the role of TxA2 is much less certain (17, 54, 57,
TxA2 and the development of these diseases (66). Findings from animal studies support the proposed role of this prostanoid in the normal state is believed to be the stimulation of platelet aggregation. Indeed, the balance between platelet-derived TxA2 and Endo-derived prostacyclin, which inhibits platelet aggregation, is crucial to the regulation of hemostasis. Recent studies in TP receptor knockout mice uphold this viewpoint, since these animals exhibit bleeding disorders but are NT (21). An exception to this rule occurs in the pulmonary vasculature where Endo-derived vasoconstrictor substances (especially TxA2) appear to play an important role in the regulation of normal vascular tone (14, 25, 63, 78).

Role of TxA2 in CV diseases. In contrast to the normal state, historically, TxA2 is believed to play a significant role in the development and/or maintenance of various CV diseases. There is clear clinical and/or experimental evidence that vascular as well as platelet TxA2 release is enhanced in a variety of CV and renal diseases, such as acute myocardial infarction, cerebral vasospasm, heart failure, diabetes, HT, preeclampsia, unstable angina, and various thrombotic disorders (12, 35, 40, 53, 54, 69). In a number of these diseases, the balance between TxA2 and prostacyclin is altered, leading to excessive vasoconstriction and hemostatic disorders. Indeed, in patients with primary vascular diseases involving excessive vasoconstriction, such as migraine headache (43), primary pulmonary HT (22), and Raynaud’s disease (68), TxA2 production is elevated, and the TxS inhibitors CGS-13080 and dazoxiben have been used successfully in the treatment of the latter two diseases (7, 66). Findings from animal studies support the proposed relationship between TxA2 and the development of these diseases (14, 26). There is also substantial evidence from both in vivo and in vitro experiments in various rat models that vascular TxA2 plays a central role in the development and/or maintenance of HT. Inhibition of TxS reduces BP and delays the onset or prevents the development of HT in the spontaneously hypertensive rat (SHR) (17) and in fructose-induced HT (31). Similarly, blockade of the TP receptor reduces or normalizes BP in two-kidney, one-clip Goldblatt HT and in ANG II-induced HT (10, 53). Chronic treatment of SHR with Ridogrel, a dual-acting TxS inhibitor/TP receptor antagonist, simultaneously reduces BP and serum TxB2 levels, as well as urinary TxB2 excretion (64). Similarly, young, pre-HT SHR exhibited exaggerated renal vascular reactivity to ANG II and TxA2 and a defect in vasodilator PG function, which may contribute to the renal vasoconstriction observed during the development of HT in this model (19, 20). These findings strongly coincide with those from isolated arteries from two-kidney, one-clip and aortic coarctation-induced HT rats, which reveal that increased release of vascular TxA2 and PGH2 is responsible for the enhanced vascular reactivity to ANG II and norepinephrine observed in these models of HT (16, 48). Thus enhanced release of TxA2 and its precursor PGH2 by the systemic vasculature appears to play an important role in pathogenesis of a number of CV diseases, especially HT.

The Role of TxA2 in Vascular Function: Historical Dogma

From the foregoing discussion, it is clear that the literature has firmly established the dogma that the constrictor prostanoids (TxA2 and PGH2), while important in pathophysiological states such as cerebral vasospasm, HT, heart failure, and unstable angina in males, play little or no role in the regulation of normal vascular tone or in females, except in the pulmonary vasculature. Indeed, in the systemic circulation, the vasodilatory prostanoid prostacyclin is the major product of the vascular wall, whereas TxA2 is produced in much lower quantities. Thus the TxA2-to-PGI2 ratio is quite low, ensuring the maintenance of local vasodilation and the inhibition of platelet aggregation and hemostatic mechanisms. Only with the activation of the platelets and the local release of significant TxA2 will the TxA2-to-PGI2 ratio be altered, stimulating platelet aggregation and local vasoconstriction. Also associated with this dogma is the role of constitutive COX-1 as the primary source of platelet-derived TxA2, whereas inducible COX-2 is the main source of Endo-derived prostacyclin (79).

As is often the case with dogmatic points of view, conflicting information is often ignored or overlooked in support of the majority opinion. In the case of constrictor prostanoids and vascular function, this involves two major points. First, most CV experimentation involving animal models predominantly and preferentially uses males; only in the last 5–10 years have most investigators become aware that the sex of the experimental animals can greatly influence the outcome of their experiments. Thus many conclusions regarding normal and pathophysiological CV mechanisms are really only applicable to males. This especially holds true for the role of TxA2 and PGH2 in the regulation of vascular tone and BP. Second, the incidences of primary vascular diseases involving excessive vasoconstriction, such as migraine headache (6, 65), primary pulmonary HT (30, 81), and Raynaud’s disease (11, 34, 80), are as much as fourfold higher in premenopausal women than in men, and excessive production of TxA2 has been implicated in the pathogenesis of these and other primary vascular diseases, especially in women (5, 8, 22, 27, 43, 68, 82). These data suggest that the ovarian steroids, probably estrogen, may be responsible for the elevated vascular TxA2 associated with these vascular diseases in women. Indeed, the ongoing studies in our laboratory on the roles of estrogen in the regulation of vascular function provide a strong challenge to the dogma regarding the role of vascular TxA2 in the regulation of vascular tone and BP in both normal and pathophysiological states.

Recent Findings on the Role of TxA2 in Vascular Function: Challenging the Dogma

Early studies from our laboratory revealed that nonselective inhibition of COX with indomethacin (Indo) attenuates contractile responses to VP in female but not in male rat aortas (Fig. 1; Ref. 73). At that time, similar findings were reported by others on the effects of Indo on vascular reactivity to phenylephrine (PE) in the female rat aorta (75) and to norepinephrine in the female rabbit aorta (52). These data suggested that sex differences in the vascular actions of some vasoconstrictors may involve agonist-induced release of constrictor prostanoids in female but not in male NT vasculature; however, the mechanisms underlying these novel but unexpected findings were not elucidated until much later. Our laboratory later reported that both Indo and the TP receptor antagonist SQ attenuated contractile responses to VP and PE to a similar extent in the NT female rat aorta (ca. 25–30%) and that ovarianectomy (Ovx) attenuated the responses to VP and PE and
A role for constrictor prostanoids in the regulation of vascular tone in NT is supported by the findings of several other recent studies. In OvX female pigs, chronic treatment with estradiol or the selective estrogen receptor modulator raloxifene enhances the plasma ratio of TxA2 to PGI2 and increases the production of contractile and proaggregatory prostanoids by femoral venous Endo and platelets, compared with Ovx. Interestingly, the constrictor prostanoid-enhancing effects of raloxifene were greater than those of estradiol (44). In aortic rings from male Wistar-Kyoto NT rats, contractions induced by the endocannabinoid 2-arachidonoyl glycerol result from VSM cell uptake of 2-arachidonoyl glycerol and its subsequent conversion to TxA2, which then causes contraction by stimu-
prostanoid mechanisms in the regulation of female vascular function in NT as well as in various other models of HT. Clearly, such studies should include an examination of these mechanisms locally in the resistance arteries and at the systemic level to fully elucidate the role of TxA2 in the regulation of female vascular function and BP.

**Perspectives**

TxA2 exerts both acute and chronic effects on the vascular wall. Historically, the acute effects of TxA2 have been related to its role as a prothrombotic agent; thus it is both a potent vasoconstrictor and a powerful activator of platelet aggregation, effects of primary importance in the initial activation of hemostasis. The vasoconstrictor action of vascular TxA2 (greater than that of ANG II) was believed to play a role mainly in pathophysiological states, such as HT and heart failure, and in males but not in females. More recent studies have highlighted the chronic effects of TxA2 to promote mitogenesis and hypertrophy of VSM and abnormal remodeling of the vascular wall. Thus TxA2 has been cast in the role of “the devil,” mediating deleterious effects on vascular function and structure. Studies from our laboratory and others challenge the dogma of vascular TxA2 and reveal that constrictor prostanoids appear to play an important role in the regulation of vascular tone and BP in the normal physiological state, as well as in a number of pathophysiological conditions, especially in the female. Furthermore, recent studies reveal a unique and unexpected deleterious effect of estrogen to potentiate constrictor prostanoid function through the upregulation of COX-2, TxA2, and TP expression, thereby enhancing vascular tone and BP.

What is the physiological relevance of the effects of “the devil” (TxA2) on normal vascular function and the action of estrogen to upregulate these deleterious effects of the constrictor prostanoids, and how is this scenario reconciled with the widely accepted dogma that estrogen plays a protective role in CV function? It is well known that estrogen upregulates the expression of Endo NO synthase (eNOS) and enhances the production of NO and other local vasodilators such as prostacyclin (18, 38, 42, 70, 85). Clearly, the balance between vasodilation and vasoconstriction is crucial to the regulation of normal vascular tone and BP in both sexes. Thus the presence of multiple, highly active, estrogen-sensitive, local vasodilator mechanisms in the female vasculature may necessitate a local counterregulatory vasoconstrictor mechanism to increase vascular tone and BP and defend against hypotension. Indeed, the coexistence of eNOS and COX-2 in caveolae (49, 60, 72) provides intriguing suggestive evidence that estrogen plays a dual role in the regulation of local dilator (NO) and constrictor (TxA2) mechanisms important in the control of normal CV homeostasis in the female and that an imbalance among these mechanisms may underlie the pathogenesis of vascular diseases involving excessive vasoconstriction, which are more common in females than in males. Thus “the devil must be paid” for the beneficial effects of estrogen to upregulate vasodilatory NO and prostacyclin pathways by its effects to enhance constrictor prostanoid function. However, based on more recent findings, we propose that these vascular actions of TxA2 and PGH2 are beneficial in the female by protecting against hypotension in the normal state, thus providing “sympathy for the devil.” Indeed, the ability of the TP receptor to suppress IP...
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and PGH2 play an important role in the regulation of normal vascular (and perhaps platelet) TxA2, which plays a key role in the initial activation of clotting mechanisms, may be central to the prothrombotic effects of estrogen.

In conclusion, present day studies on the vascular actions of TxA2 clearly challenge the dogma that constrictor prostanoids, while important in pathophysiological states, such as cerebral vasospasm, HT, heart failure, and unstable angina in males, play little or no role in the regulation of normal vascular tone in males or females. Indeed, the present data reveal that TxA2 and PGH2 play an important role in the regulation of normal vascular tone and BP, especially in the female, and that estrogen is an important regulator of constrictor prostanoid pathway function. The proposal that TxA2 serves as a local counterregulatory vasoconstrictor mechanism to maintain vascular tone and BP and defend against hypertension in the female, although tempting, must await further studies at the systemic, tissue, and molecular levels before it can be fully validated.

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