ANG II- and TxA₂-induced mesenteric vasoconstriction in rats is mediated by separate cell signaling pathways

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At present three major intracellular signal transduction pathways are recognized to be involved in transmitting receptor activation to smooth muscle contraction (11, 34): 1) mobilization of Ca²⁺ from intracellular stores via liberation of β-myoinositol 1,4,5-trisphosphate [Ins(1,4,5)P₃]; 2) transmembrane influx of Ca²⁺, predominantly via voltage-operated L-type Ca²⁺ channels; and 3) activation of one or more isoforms of protein kinase C (PKC). Intracellular Ca²⁺, increased by the first two mechanisms, binds to calmodulin to activate the Ca²⁺-calmodulin-dependent myosin light chain (MLC) kinase. The subsequent phosphorylation of a 20-kDa MLC in the smooth muscle results in an increased myosin ATPase activity and cross-bridge cycling, which initiates vasoconstriction. PKC is considered to inhibit MLC phosphatase and to render cells more sensitive to intracellular Ca²⁺ (Ca²⁺ sensitization). Furthermore, PKC has also been shown to increase intracellular Ca²⁺ via activation of L-type Ca²⁺ channels (4, 22). Even though the studies on cell signaling give insight into cellular mechanisms operative in smooth muscle cells, it should be pointed out that most of them were done in vitro preparations of vascular and nonvascular origin using phenylephrine as an agonist of choice.

Both ANG II and TxA₂ bind to specific G protein-coupled receptors with seven transmembrane domains on vascular smooth muscle cells (1, 25) to ultimately elevate smooth muscle tone by increasing phosphorylation of the MLC (11, 34). However, there are indications that the role of the above cell signaling pathways may be substantially different for TxA₂ than for ANG II or α-adrenergic agonists. In smooth muscle cells TxA₂ has been shown to increase the intracellular concentration of Ca²⁺ (10), but compared with phenylephrine, an equipotent dose of TxA₂ caused a much smaller rise in Ca²⁺. In the pulmonary circulation inhibition of L-type Ca²⁺ channels or of PKC has been reported to attenuate vasoconstriction induced by ANG II and norepinephrine but not that by TxA₂ (14, 15). Furthermore, nitric oxide, a guanylyl cyclase activator, has been found to attenuate vasoconstrictor actions of ANG II and norepinephrine in the kidney (28) and of norepinephrine in isolated vessels but not of TxA₂ (5, 8). cGMP is considered to inhibit an agonist-induced rise in intracellular Ca²⁺ and Ca²⁺ sensitization (20). Thus there might exist some causal relation between the pathogenic function of TxA₂ and peculiarities in its vascular signal transduction pathways.

The purpose of the present study was to identify which of the above signal transduction pathways are...
activated by ANG II and TxA₂ in the mesenteric circulation in vivo and to quantify their relative contribution for each agonist. For this purpose, we infused increasing doses of ANG II or the stable TxA₂ receptor agonist U-46619 into the superior mesenteric artery of anesthetized rats and determined the corresponding changes in mesenteric blood flow (MBF). The contribution of different signal transduction pathways was addressed by coinfusion of the inhibitors: Ins(1,4,5)IP₃-mediated sarcoplasmatic Ca²⁺ release by 8-diethylamino)octyl 3,4,5-trimethoxybenoate hydrochloride (TMB-8); Ca²⁺ influx by the L-type channel blocker nifedipine or by the nonspecific channel blocker Ni²⁺; and modulator effects of PKC by the PKC inhibitors staurosporine or sphingosine. All agents were infused intra-arterially to minimize their confounding systemic effects. Until recently this approach was not viable because intra-arterially applied substances are carried at an ill-defined concentration with the laminar arterial blood flow to a small and variable portion of the corresponding organ and do not allow quantitative assessment of drug effects on that organ. However, a recent technique ensuring a homogenous dispersion of the infused agents within the arterial blood (27) has overcome this problem.

**METHODS**

Experiments were performed in female Wistar rats (200–285 g) in accordance with local guidelines of animal care. The animals were deprived of food the night before the experiments but were given free access to water. After induction of anesthesia by intraperitoneal injection of 100 mg/kg 5-ethyl-5-(1-methylpropyl)-2-thio-barbituric acid (Inactin, Byk Gulden, Konstanz, Germany), the rats were placed on a thermostat table to maintain body temperature at 36–37°C. The trachea of each rat was cannulated for free breathing. Cannulas were inserted into the left femoral vein for infusion of drugs and into the left femoral artery for monitoring mean arterial pressure (MAP). For infusion of drugs into the superior mesenteric artery, we used a device developed in our laboratory for mixing the infusate thoroughly with arterial blood (27). It consists of a Teflon cannula connected to several lines for drug infusion and one line for transmitting pressure oscillations from a periodically pulsed magnetic membrane pump causing turbulence in the mesenteric artery during ejection periods. The Teflon cannula was first inserted via the right femoral artery into the abdominal aorta above the superior mesenteric artery. After the abdomen was opened by a subcostal left flank incision, the mesenteric artery and adjacent aorta were freed from surrounding tissue, and then the tip of the Teflon cannula was inserted into the mesenteric artery. A continuous infusion (2 µl/min) of heparinized saline served to keep the cannula patent. A flow probe was placed around the mesenteric artery and connected to a transit time flowmeter (T 106, Transonic System, Ithaca, NY) for measuring MBF. In all experiments, cyclooxygenase was inhibited by intravenously administered indomethacin (0.5 mg/kg bolus followed by 10 µg·kg⁻¹·min⁻¹). Experiments were begun after a 30-min stabilization period.

Experimental protocols. All experiments started with control measurements during which ANG II and U-46619 were infused intra-arterially in randomized order to reduce MBF from a threshold value to ~50% by three sequentially increasing doses. The interventions with the two agents were separated by a 10-min control period of complete recovery. In different experimental series, the measurements were repeated after intra-arterial inhibition of specific cell signaling pathways, whereby the doses of the agonists were readjusted to attain a reduction in MBF comparable to that during the control measurements. Concentrations of the inhibitors in mesenteric plasma, which could be estimated from MBF, hematocrit, and infusion rates, were adjusted to values 10 times above the reported IC₅₀ values. The inhibitors were the following: TMB-8 (0.3 µmol/min) to block intracellular Ca²⁺ release; nifedipine (3 nmol/min) to block L-type Ca²⁺ channels; Ni²⁺ (0.1 µmol/min) to block nonspecifically all Ca²⁺ channels; and staurosporine (0.5 nmol/min) or sphingosine (0.6 µmol/min) to inhibit PKC. In a separate set of experiments, the specific Txₐ receptor antagonist BM-13505 (20 nmol/min) was used to demonstrate that the response to U-46619 required activation of Txₐ receptors. Preliminary experiments revealed that different inhibitors require different pretreatment times to elicit their full effects; the time span was longest (45 min) for staurosporine and sphingosine; intermediate (20 min) for nifedipine, Ni²⁺, and BM-13505; and shortest (~3 min) for TMB-8.

Drug preparation. ANG II (Sigma Chemicals) was dissolved in isotonic saline containing 10 µM bovine serum albumin. Indomethacin (Sigma), staurosporine (Sigma), TMB-8 (RBI-Biotrend, Germany), NiCl₂ (Aldrich-Chemicals), and BM-13505 (Boehringer Mannheim, Germany) were dissolved in isotonic saline and buffered with Tris (20 mM) to give solutions appropriate for the experiments. ANG II (Sigma Chemicals) was dissolved in isotonic saline containing 10 µM bovine serum albumin. Indomethacin (Sigma), staurosporine (Sigma), TMB-8 (RBI-Biotrend, Germany), NiCl₂ (Aldrich-Chemicals), and BM-13505 (Boehringer Mannheim, Germany) were dissolved in isotonic saline and buffered with Tris (20 mM) for the first two agents. Stock solutions of U-46619 and sphingosine were made in ethanol, and before the experiment the solutions were diluted 100-fold with saline. All solutions were stored at −20°C. Nifedipine solution (Adalat, Bayer, Leverkusen, Germany) contained 18 vol% ethanol and was stored at 4°C. The vehicles for different agents did not influence the measured parameters. Concentrations of infused drugs were adjusted to obtain the required dose by infusing 10 µl/min for the inhibitors, and 3, 10, and 30 µl/min for ANG II and U-46619.

Data evaluation and statistics. Changes in MBF were normalized as percent ratios of the corresponding control values and are presented as means ± SE. To assess the shift of the dose-response curves, an ED₅₀ value was calculated for each set of measurements representing the effective dose needed to reduce MBF by 25%. This was done by interpolation between the two log dose-response data points above and below 25% MBF reduction. The ratio of ED₅₀ values before and after intervention was then used for comparison. Statistical comparisons were made by paired or unpaired t-test. P values <0.05 were considered statistically significant.

**RESULTS**

Hemodynamic effects of ANG II and U-46619. Intra-arterial infusions of ANG II and U-46619 reduced MBF in a dose-dependent manner. However, the time course of their actions was distinctly different. Whereas ANG II infusion reduced MBF to a new steady-state value within less than 1 min, the reduction due to U-46619 was slower and needed 5 min to reach the new steady-state value. After the U-46619 infusion was stopped, the recovery was also much slower than after ANG II (15 vs. 3 min). In our experiments MAP ranged under control conditions from 95 to 128 mmHg with mean values in different series of 105 to 115 mmHg, and MBF from 8 to 13 ml/min with mean values of 9.4 to 11.3 ml/min. Doses of ANG II up to 1 pmol/min, which
reduced MBF by 20–25% under control conditions, had no effect on MAP, whereas a significant rise in MAP (3–4 mmHg) was found at a dose of 3 pmol/min. Still higher doses of ANG II (10 and 30 pmol/min), which were used only in combination with different inhibitors, increased MAP by 6 and 10 mmHg, respectively. In contrast, none of the doses of U-46619 (30 to 300 pmol/min) had any effect on MAP.

In four rats the specificity of U-46619 to stimulate TxA2 receptors was tested by pretreating the mesenteric vascular bed with the TxA2 receptor antagonist BM-13505. As shown in Fig. 1, BM-13505 abolished the constrictor effect of U-46619 (100 pmol/min) but did not influence that of ANG II (1 pmol/min). Intra-arterial infusion of BM-13505, which was started after control measurements, caused a transient reduction in MBF by ∼25%, but MBF returned to the control level within 3 min of infusion.

Effects of inhibition of intracellular Ca2+ mobilization (n = 6). Intra-arterial infusion of TMB-8 had no effect on MAP (−1 ± 1 mmHg), but it increased basal MBF by 14 ± 2%. The effects of ANG II and U-46619 on MBF under control conditions and following the inhibition of intracellular Ca2+ mobilization by TMB-8 are shown in Fig. 2. TMB-8 shifted the dose of ANG II needed to reduce MBF by 25% to a 2.9 ± 0.2-fold higher value (ED25, 3.32 ± 0.28 vs. 1.16 ± 0.09 pmol/min). In contrast, TMB-8 had no effect on ED25 of U-46619 (101 ± 6 vs. 104 ± 7 pmol/min).

Effects of subsequent inhibition of Ca2+ channels and Ca2+ mobilization (n = 5). The Ca2+ channel blocker nifedipine decreased MAP by 10 ± 2 mmHg and increased MBF by 20 ± 3%. Nifedipine (Fig. 3) increased the ED25 of ANG II 2.7 ± 0.6-fold (3.06 ± 0.61 vs. 1.12 ± 0.06 pmol/min) but did not affect that for U-46619 (90 ± 12 vs. 103 ± 9 pmol/min). Additional infusion of TMB-8 further increased MBF by 12 ± 2% without affecting MAP and increased the ED25 value for ANG II 3.2 ± 0.8-fold (8.68 ± 1.80 vs. 3.06 ± 0.61 pmol/min). This maneuver had also no effect on U-46619 (ED25, 93 ± 12 vs. 90 ± 12 pmol/min).

Five additional experiments were done to compare the modulator effects of nonspecific Ca2+ channel blockade by Ni2+ with that of specific blockade by nifedipine. Infusion of Ni2+ increased MBF (21 ± 2%) to the same extent as nifedipine, but in contrast to nifedipine, it tended to increase MAP (4 ± 1 mmHg). The right shift of the dose-response curve of ANG II by Ni2+ to a 3.9 ± 0.6-fold higher value (ED25, 4.88 ± 0.63 vs. 1.23 ± 0.11 pmol/min) was not significantly different from that observed for nifedipine. Ni2+ did not alter the ED25 value for U-46619 (95 ± 12 vs. 95 ± 8 pmol/min).

**Fig. 2.** Effects of inhibition of intracellular Ca2+ mobilization: relationships between intramesenteric doses of ANG II and U-46619 and the respective changes in MBF under control conditions (open circles) and during inhibition of intracellular Ca2+ release by TMB-8 (closed circles). Effective dose (ED25) of ANG II shifted after TMB-8 administration to a 2.9-fold higher value (P < 0.05), but that of U-46619 was not affected.
Effects of subsequent inhibition of PKC and Ca\textsuperscript{2+} mobilization (n = 6). The PKC inhibitor staurosporine reduced MAP by 6 ± 1 mmHg and increased MBF by 13 ± 2%. The effects of staurosporine on the dose-response curves are shown in Fig. 4. Staurosporine increased the ED\textsubscript{25} of ANG II 3.4 ± 0.3-fold (4.26 ± 0.23 vs. 1.29 ± 0.08 pmol/min) but did not affect that for U-46619 (113 ± 13 vs. 97 ± 8 pmol/min). Additional infusion of TMB-8 increased MBF further by 12 ± 1% without altering MAP. The ED\textsubscript{25} value for ANG II increased 3.3 ± 0.4-fold (13.50 ± 1.05 vs. 4.26 ± 0.23 pmol/min), whereas that for U-46619 did not change (111 ± 10 vs. 113 ± 13 pmol/min).

In five experiments, we tested the effect of sphingosine, a structurally different PKC inhibitor, using single doses of ANG II and U-46619. In contrast to staurosporine, sphingosine had no significant effects on basal MAP and MBF. However, like staurosporine it diminished the MBF reduction elicited by ANG II (1 pmol/min) from 21.2 ± 2.1% to 9.0 ± 1.9% and did not influence the effect of U-46619 (100 pmol/min, 24.2 ± 1.9 vs. 25.0 ± 2.2%).

Effects of subsequent inhibition of PKC, Ca\textsuperscript{2+} channels, and Ca\textsuperscript{2+} mobilization (n = 5). In these experiments, staurosporine decreased MAP by 5 ± 1 mmHg and increased MBF by 17 ± 2%. As shown in Fig. 5, staurosporine increased the ED\textsubscript{25} of ANG II 3.4 ± 0.6-fold (4.18 ± 0.98 vs. 1.21 ± 0.04 pmol/min) but did not affect that of U-46619 (112 ± 11 vs. 100 ± 8 pmol/min). Subsequent infusion of nifedipine caused an additional decrease in MAP by 11 ± 2 mmHg and a increase in MBF by 20 ± 3%. The ED\textsubscript{25} of ANG II increased further 4.3 ± 0.7-fold (16.94 ± 2.98 vs. 4.18 ± 0.98 pmol/min), whereas that of U-46619 remained unchanged (110 ± 7 vs. 112 ± 11 pmol/min). Additional infusion of TMB-8 increased MBF by 11 ± 3%. Thereafter, no significant reduction in MBF could be achieved with ANG II up to a dose of 30 pmol/min, which increased MAP by 8 ± 2 mmHg. At a dose of 100 pmol/min, ANG II even tended to increase MBF along with a rise in MAP (>13 mmHg) and the infusion was stopped. Intra-arterial infusion of all three inhibitors had no effect on the dose-response curve of U-46619. The ED\textsubscript{25} value of 109 ± 10 pmol/min was not significantly different from the initial control value of 100 ± 8 pmol/min.

**DISCUSSION**

The results of this study demonstrate that ANG II induces mesenteric vasoconstriction by a combined action of intracellular Ca\textsuperscript{2+} release, Ca\textsuperscript{2+} influx through

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Fig. 4. Effects of inhibition of protein kinase C (PKC): relationships between intramesenteric doses of ANG II and U-46619 and respective changes in MBF (n = 6 rats) under control conditions (open circles) and during inhibition of PKC by staurosporine (closed triangles). Effective dose (ED\textsubscript{25}) of ANG II shifted after staurosporine administration to a 3.4-fold higher value (P < 0.05), but that of U-46619 was not affected.
L-type Ca\textsuperscript{2+} channels, and activation of PKC. Each signal transduction pathway contributes to the vasoconstriction to about the same degree. The action of U-46619 is mediated by additional, still unidentified, signal transduction pathways, which are different from those activated by ANG II.

The importance of Ca\textsuperscript{2+} mobilization, Ca\textsuperscript{2+} influx, and activation of PKC for ANG II-mediated vasoconstriction has been demonstrated previously. Attenuation of ANG II-induced vasoconstriction by TMB-8 and nifedipine has been shown in the mesentery and kidney of dogs (35) and in the rat kidney (31). An important role of L-type Ca\textsuperscript{2+} channels has further been demonstrated in the mesenteric and pulmonary circulations in cats (15, 21). Participation of PKC has been shown in the rat kidney (32, 33) and in the cat lung (14).

Our results confirm the above findings for the mesenteric circulation in rats. In contrast to the above-cited studies, which employed intra-arterial bolus injections, we used continuous intra-arterial infusions to assess the vascular response to ANG II. Our data further indicate that Ca\textsuperscript{2+} channels, other than L-type channels, do not play an important role, because both nifedipine and the nonselective Ca\textsuperscript{2+} blocker Ni\textsuperscript{2+} reduced the ANG II effect on MBF to a similar extent. This conclusion is supported by the observation that additional administration of Ni\textsuperscript{2+} after nifedipine had no further effect on ANG II-mediated vasoconstriction (n = 3, data not shown). Our results (Fig. 5) also suggest rather independent roles of Ins(1,4,5)P\textsubscript{3}, Ca\textsuperscript{2+} channels, and PKC for ANG II-induced vasoconstriction, because effects of subsequent blockade of different pathways were additive. In vitro studies have suggested that PKC elicits a part of its constrictor effect via opening of L-type Ca\textsuperscript{2+} channels (4, 22). The present results, however, do not support this notion for an in vivo situation, because the efficacy of nifedipine was not attenuated after blockade of PKC by staurosporine. Our data also provide circumstantial evidence for roles of the above pathways for basal tone of mesenteric vessels. Intra-arterial infusion of each of the inhibitors TMB-8, nifedipine, and staurosporine increased basal MBF to a comparable degree (~15%). In the experiments using all three inhibitors, the total rise in MBF was >50%.

The present study excludes substantial roles of an increase in intracellular Ca\textsuperscript{2+} and an activation of PKC for the vascular effect of TxA\textsubscript{2} in mesenteric circulation in vivo. The reduction in MBF induced by the TxA\textsubscript{2} agonist U-46619 was not affected by TMB-8, nifedipine, Ni\textsuperscript{2+}, or staurosporine. Also the combination of the antagonists, which abolished effects of ANG II, did not influence efficacy of U-46619. Though both ANG II and TxA\textsubscript{2} receptors are seven transmembrane domain proteins coupled to G proteins, they apparently activate independent signaling pathways.

Previous in vitro studies have investigated the role of Ca\textsuperscript{2+} and PKC in mediating vasoconstrictor effects of TxA\textsubscript{2} with variable results. In porcine coronary artery preparations, Bradley and Morgen (3) showed that a vasoconstriction induced by U-46619 was not associated with a rise in intracellular Ca\textsuperscript{2+}. Himpens et al. (10) did find a rise in Ca\textsuperscript{2+} in the rabbit pulmonary artery constricted with U-46619, but the rise was much smaller than that induced by an equipotent dose of phenylephrine. Also the Ca\textsuperscript{2+} sensitization due to U-46619 was much larger than that with phenylephrine. Dorn and Becker (7) found that the constrictor response of U-46619 in rat aortic rings was not affected by the removal of extracellular Ca\textsuperscript{2+} but was attenuated after depletion of intracellular Ca\textsuperscript{2+} stores. Kurata et al. (18) found a fast and a slow component of contraction by U-46619 in the rat aorta. Removal of extracellular Ca\textsuperscript{2+} abolished only the fast component, whereas the slow component was independent of calcium influx, calcium mobilization [Ins(1,4,5)P\textsubscript{3}], and PKC activation. Jiang et al. (12) reported that in guinea pig aortic strips, U-46619-induced contraction was attenuated by removal of extracellular Ca\textsuperscript{2+} and by inhibition of PKC. In the human intrapulmonary artery, the TxA\textsubscript{2} effect was diminished by inhibitors of phospholipase C, indicating an involvement of Ins(1,4,5)P\textsubscript{3} and PKC (13). Hayashi et al. (9) reported that in the isolated perfused hydronephrotic kidney, nifedipine or diltiazem blunted the afferent but not efferent arteriolar constriction induced by the TxA\textsubscript{2}.
mimetic U-44069. These studies do imply some role of Ca$^{2+}$ and PKC in TXA$_2$-induced vasoconstriction but not as unequivocally as for ANG II or norepinephrine.

Kadowitz and co-workers (6, 14, 15, 21, 24) have investigated cell signaling pathways for different vasoconstrictors, including U-46619 in the pulmonary and mesenteric vascular bed in cats in vivo. Vasoconstriction induced by the bolus injection of U-46619 into a pulmonary lobar artery was not influenced by inhibitions of L-type Ca$^{2+}$ channels, phospholipase C, and PKC, whereas all inhibitors reduced responses to ANG II and norepinephrine (14, 15). Our results from the rat mesentery are in accordance with their results. In the mesenteric circulation, however, they found some attenuation of the U-46619 effect by nifedipine, which was less pronounced than that for ANG II and norepinephrine (21). How far this difference is related to different species or the mode of drug administration (bolus vs. continuous infusion) remains open. Whereas in our experiments plateau reductions in MBF by U-46619 were obtained after an infusion of about 5 min, the bolus injections in their experiments caused maximum changes within less than 1 min. In analogy to the results from the cat lung (14, 15), we also observed in our preliminary experiments ameliorating effects of TMB-8, nifedipine, and staurosporine on mesenteric vasoconstriction induced by norepinephrine. Discrepancies between in vitro and in vivo data can be reconciled by considering limitations inherent to in vivo preparations. Even with intra-arterial infusion, any of the cell signaling pathway can be inhibited only to a certain degree without producing large systemic effects. Also rigorous maneuvers such as the removal of extracellular Ca$^{2+}$ are not possible. However, they allow us to differentiate the relative role of a pathway for different agonists, which is still relevant for practical purposes.

Although the mechanisms of action of TXA$_2$ remain unclear, a phosphorylation of MLC seems to be involved. A MLC kinase inhibitor has been shown to inhibit the U-46619 effect in the cat pulmonary circulation (15). A putative signaling pathway for TXA$_2$ may involve Ca$^{2+}$ sensitization without an involvement of PKC. Recent studies have shown a PKC-independent mechanism for Ca$^{2+}$ sensitization via activation of rho-associated kinase. An important participation of changes in the activity of the small G protein rho, which regulates the rho-associated kinase, in the process of Ca$^{2+}$ sensitization has been demonstrated in vitro (17, 19, 23). The first evidence for a distinct role of rho kinase in TXA$_2$-mediated vasoconstriction has been put forward recently. In strips of the pig coronary artery and the guinea pig trachea, the rho kinase inhibitor Y-27632 was found to antagonize the U-46619 response (36). In preliminary experiments we found that Y-27632 antagonized the vasoconstrictor response to U-46619 in the mesenteric circulation. Another possible mechanism may be that TXA$_2$ has a vasodilator component counterbalancing a part of its vasoconstrictor component, both using similar pathways in a complex fashion. This possibility is supported by the findings of the Kadowitz group (24), namely that the vasodilator response of a cGMP-dependent phosphodiesterase (PDE-5) inhibitor was enhanced in lungs preconstricted with U-46619 and that the pulmonary effects of U-46619 were accentuated by an inhibitor of an ATP-dependent K$^+$ channel (6).

It is interesting to note that ANG II, which can also be involved in pathogenic processes, displays a significant part of its pathophysiological effects by enhancing the synthesis of TXA$_2$. For example, TXA$_2$ inhibition attenuated the reduction in renal blood flow, which had been induced by an elevation of plasma ANG II levels for 5 days (16). Similar processes have been described to contribute significantly to the development and maintenance of Goldblatt hypertension (2). Furthermore, pulmonary vascular effects of serotonin, also a spasmyogenic agent like TXA$_2$, were not influenced by inhibition of PKC and marginally attenuated by inhibition of Ca$^{2+}$ channels or phospholipase C in cats (14, 15). Thus it is conceivable that pathogenic vasoconstrictors have some common features regarding their cell signaling pathways. Understanding the intracellular signaling cascades for TXA$_2$ and other related hormones could therefore be of considerable clinical relevance.

In conclusion, the present data demonstrate relatively independent cell signaling pathways for ANG II and TXA$_2$ for mesenteric vessels in vivo. The vasoconstriction induced by ANG II is mediated to similar extents via three mechanisms, namely Ca$^{2+}$ mobilization, Ca$^{2+}$ influx, and activation of PKC. Though the corresponding cascade for TXA$_2$ remains as yet unknown, its future elucidation seems rewarding.

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