P2Y₂ receptor activation decreases blood pressure and increases renal Na⁺ excretion

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Rieg T, Gerasimova M, Boyer JL, Insel PA, Vallon V. P2Y₂ receptor activation decreases blood pressure and increases renal Na⁺ excretion. Am J Physiol Regul Integr Comp Physiol 301: R510–R518, 2011. First published May 25, 2011; doi:10.1152/ajpregu.00148.2011.—ATP and UTP are endogenous agonists of P2Y₂/4 receptors. To define the in vivo effects of P2Y₂ receptor activation on blood pressure and urinary excretion, we compared the response to INS45973, a P2Y₂ agonist, and ATP, in wild-type (WT) and P2Y₂ receptor knockout (P2Y₂⁻/⁻) mice. INS45973 was administered intravenously as a bolus injection or continuous infusion to determine effects on blood pressure and renal function, respectively. Within seconds, bolus application of INS45973 (0.1 to 3 mg/kg body wt) dose-dependently decreased blood pressure in WT (maximum response 18 ± 1 mmHg) but returned to basal levels within 60 s. Continuous infusion of INS45973 (25 to 750 μg·min⁻¹·kg⁻¹ body wt) dose-dependently increased urinary excretion of Na⁺ in WT (maximum response 46 ± 15%) but reduced Na⁺ excretion in P2Y₂⁻/⁻ (maximum responses of 45 ± 15%) mice. In renal clearance experiments, INS45973 affected the fractional excretion of fluid, Na⁺, and K⁺ in WT relative to P2Y₂⁻/⁻ mice. The blood pressure responses to INS45973 are consistent with P2Y₂ receptor-mediated NO-independent vasodilation and implicate responses to endothelium-derived hyperpolarizing factor, and P2Y₂ receptor-independent vasoconstriction, probably via activation of P2Y₂ receptors on smooth muscle. Systemic activation of P2Y₂ receptors thus lowers blood pressure and inhibits renal Na⁺ reabsorption, effects suggesting the potential utility of P2Y₂ agonism in the treatment of hypertension.

The vasodilator response to ATP is abolished by mechanical removal of the endothelium, resulting in a vasoconstrictor response via an effect of ATP on the vascular smooth muscle (19, 37). This response is consistent with the expression of P2Y or P2X receptor subtypes on vascular smooth muscle cells that promote contraction in response to ATP by increasing cytosolic Ca²⁺ (4, 6, 9, 20). Among P2Y receptors, subtypes P2Y₂/4/6 have been identified on vascular smooth muscle cells of rats and mice (10, 18). In intact rat arteries, activation of the P2Y₄ receptor is associated with vasoconstriction (12, 28, 47).

Opposing effects on endothelium and smooth muscle may explain why vascular effects of ATP and UTP can vary (35). While ATP induces vasodilation, UTP promotes vasoconstriction in rabbit ear artery and mouse aortic rings (3, 18, 56). Other studies have reported that ATP and UTP can cause vasodilation, vasoconstriction, or both, depending on species, vessel type, location within the vascular tree, and/or route of administration (5, 7, 14, 15, 36, 37, 39).

Recently, we obtained in vivo evidence for a physiological role of P2Y₂ receptors in blood pressure regulation by demonstrating that P2Y₂⁻/⁻ mice have salt-resistant arterial hypertension (41). In the kidney, P2Y₂ receptors have an inhibitory influence on Na⁺ and fluid reabsorption (33, 44, 54, 60). Activation of P2Y₂ receptors in the apical membrane of the aldosterone-sensitive distal nephron (ASDN) inhibits the open probability (Pₒ) of the epithelial sodium channel (ENaC), an effect mediated by activation of phospholipase C (31, 32). In addition, we showed that an increase in the apical release of ATP and/or UTP, which activates P2Y₂ receptors, mediates the suppression of ENaC Pₒ in response to increasing salt intake, thereby contributing to NaCl homeostasis and blood pressure regulation (32). We also found that lack of P2Y₂ receptors is associated with increased activity of Na⁺–K⁺–2Cl⁻ cotransporter isofrom 2 (NKCC2) in the thick ascending limb and increased water reabsorption in the collecting duct (41).

To further define the in vivo role of P2Y₂ receptors and potentially that of P2Y₄ receptor activation, in the current studies, we compared the effects of systemic administration of a P2Y₂/4 receptor agonist (INS45973) on blood pressure and renal excretion in wild-type (WT) and P2Y₂⁻/⁻ mice. We find that such administration acts via P2Y₂ receptor activation to lower blood pressure, an effect that is independent of endothelial NO, and to inhibit renal Na⁺ reabsorption. Acute blood pressure responses to INS45973 in mice lacking P2Y₂ receptors imply that P2Y₄ receptor activation may increase blood pressure.

MATERIALS AND METHODS

Animal experiments were conducted according to the protocols reviewed and were approved by the Institutional Animal Care and Use Committee of the Veterans Affairs San Diego Healthcare System.
Generation of P2Y$_2$–/– mice has been described earlier (11). P2Y$_2$–/– mice were backcrossed to 129SvJ for a total of seven generations. Heterozygous animals from the final backcross were bred to each other, so as to yield P2Y$_2$–/– and littermate WT mice. Genotyping was done by polymerase chain reaction from ear tissue DNA and use of primers, as previously described (41). Endothelial nitric oxide synthase knockout mice (eNOS$^{-/-}$), were purchased from the Jackson Laboratory (Bar Harbor, ME). Adult male mice were used for all experiments.

INS45973, P1-(inosine 5'')P$_4$-(uridine 5'')tetraphosphate tetrasodium salt, or Ip$_4$U$_4$ Na$^+$ (Raleigh, NC). INS45973 has EC$_{50}$ values for P2Y$_2$ and P2Y$_4$ of 2.25% BSA in 0.85% NaCl at a rate of 0.4 ml·h$^{-1}$, consistent with the short half-life of INS45973.

The femoral artery was cannulated for blood pressure measurement. A femoral vein was cannulated for continuous infusion and 100% oxygen was blown toward the tracheal tube throughout the experiment. The trachea was cannulated, and 100% oxygen was blown toward the tracheal tube throughout the experiment. The jugular vein was cannulated for continuous infusion of 2.25% BSA in 0.85% NaCl at a rate of 0.4 ml·h$^{-1}$. The femoral artery was cannulated for blood pressure measurement. A catheter was placed in the bladder to drain the urine. After surgery, mice were allowed 60 min to stabilize before experiments were started. Acute blood pressure responses were monitored following the application of vehicle (1 µg/g body wt of 0.85% NaCl) or INS45973 (0.1, 0.3, 1, or 3 mg/kg body wt in increasing doses), which were given intravenously over 25 s. We allowed a 10-min time interval between applications.

**Experiment 2, urinary excretion experiments.** Animals were prepared as described above. Urinary excretion of fluid, Na$^+$ and K$^+$ were assessed by quantitative urine collection via the bladder catheter in response to vehicle or INS45973, which were administered by continuous infusion (25, 75, 250, or 750 µg·min$^{-1}$·kg$^{-1}$ body wt iv for 15 min in 0.4 ml·h$^{-1}$.naCl body wt). Quantitative urine collections were performed over the last 10 min. Urinary volume was determined gravimetrically, and flow rate was calculated on the basis of collection time. Urine was analyzed for Na$^+$ and K$^+$ concentrations by flame photometry (Cole-Parmer Instrument, Vernon Hills, IL).

**Experiment 3, two-period clearance experiments to assess glomerular filtration rate and renal reabsorption.** Animals were prepared as described above. For assessment of two-kidney glomerular filtration rate (GFR) by inulin clearance, [H]$^3$H]inulin was added to the infusion to deliver 5 µCi·h$^{-1}$·30$^{-1}$ g·min body wt at an infusion rate of 0.4 ml·h$^{-1}$.30$^{-1}$ g·body wt, followed by two 30-min periods of urine collection: a basal period (P1) and a subsequent period (P2). After completion of P1 and 5 min before starting P2, INS45973 was added to the infusion at a dose of 250 µg·min$^{-1}$·kg$^{-1}$ body wt (found to be natriuretic in experiment 2). Blood samples (50 µl) were drawn midway in each period from the arterial catheter. Blood pressure and heart rate were continuously monitored. Concentrations of [H]$^3$H]inulin in plasma and urine were measured by liquid scintillation counting. Plasma and urine were analyzed for Na$^+$ and K$^+$ concentrations by flame photometry.

![Fig. 1](http://ajpregu.physiology.org/). Representative original recordings of the blood pressure (BP) response to INS45973 in a wild-type (WT) and a P2Y$_2$ knockout mouse (P2Y$_2$–/–). In WT, application of INS45973 dose-dependently and rapidly decreased blood pressure, which started to partially recover during drug application. In contrast, INS45973 in P2Y$_2$–/– dose-dependently and rapidly increased blood pressure, which was sustained during drug application and thereafter recovered to baseline within 1–2 min, consistent with the short half-life of INS45973.
**Statistical analysis.** The data are expressed as means ± SE. Unpaired and paired t-tests were performed, as appropriate, to analyze for statistical differences between and within groups. *P* ≤ 0.05 vs. WT and *P* ≤ 0.05 vs. vehicle were considered statistically significant. The contribution of P2Y2 in the response to INS45973 was determined by comparing changes in WT vs. P2Y2−/− mice.

**RESULTS**

**Experiment 1, acute blood pressure and heart rate response to INS45973 in WT, P2Y2−/−, and eNOS−/− mice.** Blood pressure recordings in WT and P2Y2−/− mice in response to INS45973 are illustrated in Fig. 1. Application of vehicle...
had no effect on blood pressure or heart rate in either genotype. Acute application of INS45973 in WT dose-dependently and rapidly (within 15 s of starting infusion) decreased blood pressure (maximum response $-35 \pm 2$ mmHg, Figs. 1 and 2). Notably, blood pressure began to increase toward basal levels, while the bolus was being administered. In contrast, INS45973 induced a dose-dependent, rapid (within 15 s of starting infusion) increase in blood pressure in P2Y2$^-$/- mice (maximum response $+18 \pm 1$ mmHg) that was sustained during drug administration but then returned to baseline within $\sim$1 min (Figs. 1 and 2), consistent with the short half-life of the drug. INS45973 did not significantly change heart rate in either genotype (Fig. 2), perhaps because of blunted baroreceptor response under barbiturate anesthesia (22). Basal heart rate was not different between genotypes (WT: 495 $\pm$ 14 vs. 489 $\pm$ 19 min$^{-1}$), and basal mean arterial blood pressure was similar in P2Y2$^-$/- and WT mice (111 $\pm$ 4 vs. 108 $\pm$ 5 mmHg), the latter result contrasts with our previous observations (32, 41). A possible explanation is an influence of the genetic background (129Sv/J in current vs. C57BL/6J in previous studies).

In eNOS$^-$/- mice, basal mean arterial blood pressure and heart rate were 131 $\pm$ 3 mmHg and 479 $\pm$ 17 min$^{-1}$, respectively. Bolus administration of INS45973 dose-dependently and rapidly decreased blood pressure, similar to the response of WT mice (maximum response $-42 \pm 2$ mmHg). In contrast to WT and P2Y2$^-$/- mice, however, eNOS$^-$/- mice had a biphasic response with a pronounced increase in blood pressure above basal values (maximum response $+22 \pm 3$ mmHg) immediately following the initial decrease (Fig. 3 and 4). Heart rate was unaffected by application of INS45973 (WT vs. P2Y2$^-$/- mice).

Experiment 2, urinary excretion in response to continuous infusion of INS45973 in WT and P2Y2$^-$/- mice. Under basal conditions, urinary flow rate and Na$^+$ and K$^+$ excretion were not different between WT and P2Y2$^-$/- mice (Table 1). In WT mice, INS45973 dose-dependently increased urinary Na$^+$ excretion (Fig. 5, maximum response $+46 \pm 15\%$), but the excretion of fluid and K$^+$ was similar to basal measurements (Fig. 5). In P2Y2$^-$/- mice, INS45973 reduced urinary excretion of Na$^+$, K$^+$, and fluid compared with basal values (Fig. 5, maximum responses of $-45 \pm 15\%$, $-50 \pm 6\%$, and $-37 \pm 7\%$, respectively). The responses in urinary excretion of Na$^+$, K$^+$, and fluid to INS45973 were significantly different between genotypes.

**Experiment 3, blood pressure, GFR, and fractional urinary excretion in response to continuous infusion of INS45973 in WT and P2Y2$^-$/- mice.** To test whether changes in urinary excretion were caused by changes in GFR, two-period clearance experiments were performed. A dose of 250 $\mu$g INS45973·min$^{-1}$·kg$^{-1}$ body wt was chosen on the basis of its natriuretic effect observed in experiment 2. Figure 6 shows responses of WT and P2Y2$^-$/- mice to INS45973. Similar to findings in experiment 1, a continuous infusion of INS45973 decreased blood pressure in WT but not P2Y2$^-$/- mice and produced significantly different responses between genotypes (Fig. 6, A and B). Arterial hematocrit was identical between genotypes (WT: 49 $\pm$ 1% vs. P2Y2$^-$/-: 49 $\pm$ 1%), decreasing slightly in the second period and to a similar extent in WT (48 $\pm$ 1% and P2Y2$^-$/-: 47 $\pm$ 1%) mice. No significant change in heart rate occurred (WT vs. P2Y2$^-$/- basal: 498 $\pm$ 22 vs. 493 $\pm$ 14 min$^{-1}$, INS45973: 495 $\pm$ 24 vs. 503 $\pm$ 26 min$^{-1}$). GFR was similar between WT and P2Y2$^-$/- mice under basal

| Table 1. Basal parameters in wild-type and P2Y2$^-$ receptor knockout mice |
|---------------------------------|-----------------|
| **WT (n = 7)**                  | **P2Y2$^-$/- (n = 8)** |
| Body weight, g                  | 27 $\pm$ 1      | 30 $\pm$ 1      |
| Hematocrit, %                   | 49 $\pm$ 1      | 49 $\pm$ 1      |
| Urinary flow rate, nl·min$^{-1}$·g$^{-1}$ body wt | 40 $\pm$ 8      | 44 $\pm$ 5      |
| $U_{Na}V$, nmol·min$^{-1}$·g$^{-1}$ body wt | 6 $\pm$ 1       | 7 $\pm$ 1       |
| $U_kV$, nmol·min$^{-1}$·g$^{-1}$ body wt | 7 $\pm$ 1       | 9 $\pm$ 1       |

Values are expressed as means $\pm$ SE. WT, wild-type; P2Y2$^-$/-, P2Y2 receptor knockout mice.
conditions (8.7 ± 1 vs. 9.5 ± 1 μl·min⁻¹·g⁻¹ body wt; not significant) and did not change significantly during infusion of INS45973 (8.1 ± 1 vs. 9.6 ± 1 μl·min⁻¹·g⁻¹ body wt). Despite the decrease in blood pressure in WT, INS45973 increased absolute and fractional excretion of H₂O, Na⁺, and K⁺. By contrast, INS45973 decreased absolute and fractional H₂O, Na⁺, and K⁺ excretion in P2Y₂⁻/⁻ mice. Thus, the changes in absolute and fractional excretion of Na⁺ and K⁺ were opposite and significantly different between genotypes.

Fig. 5. Responses in urinary excretion of fluid (A), Na⁺ (B), and K⁺ (C) to continuous infusion of INS45973 in wild-type (WT, n = 7) and P2Y₂ knockout mice (P2Y₂⁻/⁻, n = 8). The responses in urinary excretion of fluid, Na⁺ and K⁺ excretion in response to continuous infusion of INS45973 for 15 min were significantly different between WT and P2Y₂⁻/⁻ mice, as determined over the last 10 min. *P < 0.05 vs. WT.

Fig. 6. Response in blood pressure, GFR, and renal reabsorption to continuous infusion of INS45973 (250 μg·min⁻¹·kg⁻¹ body wt) in wild-type (WT, n = 5) and P2Y₂ knockout mice (P2Y₂⁻/⁻, n = 5) in two-period clearance experiments. INS45973 decreased blood pressure (A and B) in WT mice, which was different from the response in P2Y₂⁻/⁻ mice. C: INS45973 did not alter GFR in either genotype. WT and P2Y₂⁻/⁻ mice showed opposite responses to INS45973 in fluid (D and G), Na⁺ (F and H), and K⁺ (E and I) excretion, such that responses in absolute and fractional Na⁺ and K⁺ excretion were significantly different between genotypes. *P < 0.05 vs. WT.
DISCUSSION

Little is known about the responses in blood pressure and renal function to acute systemic activation of P2Y2 and P2Y4 receptors. Here, we used INS45973, a UTP analog and P2Y2/4 receptor agonist, to gain new insights regarding these responses. To our knowledge, these are the first studies to provide direct in vivo evidence that acute activation of P2Y2 receptors decreases blood pressure in a manner that is independent of endothelium-derived NO. Notably, the absence of P2Y2 receptors unmasks an acute increase in blood pressure in response to INS45973, an effect that may be mediated by P2Y4 receptor activation. Both the decrease in blood pressure in WT mice and the increase in P2Y2−/− mice likely result from changes in peripheral resistance, since the effects occurred within seconds of agonist application and in the absence of changes in heart rate.

Our findings are in agreement with previous studies that showed a dose-dependent decrease in arterial blood pressure in anesthetized mice with acute intravenous administration of ATP and UTP (50). The authors used pharmacological agents to conclude that P2X1, P2Y1 (both not activated by UTP) and ATP and UTP (50). The authors used pharmacological agents to conclude that P2X1, P2Y1 (both not activated by UTP) and P2Y4 receptors did not significantly contribute to this response and surmised that P2Y2 receptors potentially mediated vasodilation. Consistent with this finding, Guns et al. (8) showed that ATP-evoked relaxation was impaired in the aorta of P2Y2−/− mice. By contrast, ATP- and UTP-evoked vasoconstriction was intact in aortas of P2Y4−/− mice (7). Conclusions from these latter two studies on blood pressure, however, were hampered by the fact that the aorta is not considered a resistance vessel.

How does P2Y2 receptor activation cause vasodilation in WT mice? It has been shown that endothelial P2Y2 receptors stimulate the synthesis and release of NO (34–37). Thus, we studied the effect of INS45973 in eNOS−/− mice. We found that eNOS−/− mice show the same rapid blood pressure decrease in response to INS45973, as do WT mice, results that imply a role for the synthesis and release of endothelium-derived hyperpolarization factor (EDHF). On the basis of results from in vitro studies, it has been speculated that EDHF is a major mediator for dilation of the peripheral vascular bed by nucleotides (24, 27). Moreover, UTP-mediated reduction of vascular resistance in the human forearm is resistant to eNOS blockade (13, 49). Our studies showing that P2Y2 receptor activation decreases blood pressure in an eNOS-independent manner are thus consistent with a role for EDHF in mediating vasodilation.

Stimulation of P2Y2 and P2Y4 receptors on vascular smooth muscle cells produces contraction (53), and UTP constricts murine pial arterioles in vivo, independent of the endothelium (45, 46). In porcine coronary arteries, suramin reduces UTP-promoted vasoconstriction; the authors concluded that P2Y2 and/or P2Y4 receptors mediate(s) this response (39). In rat arteries, P2Y4 receptors have been associated with contractile effects (12, 20, 47). The conclusions regarding P2 receptor subtypes in these studies relied on the use of pharmacological tools, e.g., UTP or Up4A as agonists of P2Y2/4 receptors (17) in the presence or absence of nonspecific antagonists [e.g., suramin, reactive blue 2, or pyridoxal-phosphate-6-azophenyl-2, 4-disulphonic acid (PPADS)]. Data from the current studies in which we used the P2Y2/4 agonist INS45973 in WT and eNOS knockout mice (7). Conclusions from these latter two studies on blood pressure, however, were hampered by the fact that the aorta is not considered a resistance vessel.

INS45793 increases blood pressure by inducing P2Y2 receptor-mediated endothelial NO-independent vasodilation, implicating a role for endothelial derived hyperpolarization factor (EDHF) (1). Activation of smooth muscle P2Y4 receptors results in vasoconstriction (2), which is counterbalanced by P2Y4 receptor-induced endothelial NO formation and release (3). Middle: in P2Y2 receptor knockout mice INS43793 decreases blood pressure by activating P2Y4 receptors on smooth muscle cells (2), which is counterbalanced by NO release following endothelial P2Y4 receptor activation (3). Right: in eNOS−/− mice, INS43793 decreases blood pressure via P2Y2 receptor-induced EDHF (1). The increase in blood pressure due to P2Y4 receptor activation on smooth muscle cells (2) is enhanced and sustained compared with wild-type and P2Y2 receptor knockout mice due to the lack of P2Y4 receptor-induced NO release from endothelial cells.
present and have similar affinities for INS45973, P2Y2 receptor-mediated vasodilation is more prominent than P2Y4 receptor-promoted vasoconstriction. However, spatial and temporal target accessibility of endothelial cells and vascular smooth muscle cells to the drug could differ. In this regard, bolus application of INS45973 in WT mice rapidly and strongly decreased blood pressure, a response that partially recovered during drug administration (Fig. 1), perhaps reflecting an opposing action of P2Y4 receptors on vascular smooth muscle cells. Intravenously administered INS45973 should reach P2Y2 receptors on endothelial cells before P2Y4 receptors on smooth muscle cells, but vasodilation in response to activation of the former may require the release and action of an endothelial-derived vasodilator.

Our studies in eNOS−/− mice indicate that the initial P2Y2 receptor-promoted decrease in blood pressure is not mediated by endothelial NO but may involve EDHF formation, release, and action. Deficiency of eNOS unmasked a more prominent, sustained increase in blood pressure in response to INS45973 than the lack of P2Y2 receptors. We speculate that endothelial release of NO induced by activation of endothelial P2Y4 receptors may balance the vasoconstriction produced by P2Y4 receptor activation on smooth muscle cells. Further in vivo studies are needed to quantify blood pressure effects of ATP, UTP, and their analogs in P2Y4 receptor knockout mice to test that idea and also to test whether endothelial P2Y2 and P2Y4 receptors induce different responses via EDHF and eNOS, respectively.

Anesthetized P2Y2−/− mice on a Sv129 genetic background had similar blood pressure as did WT mice, whereas blood pressure was significantly greater in knockouts if mice were on a C57BL/6J genetic background (32, 41). The importance of the genetic background in terms of blood pressure is a well-described phenomenon in mice (48) and occurs for other genes [e.g., bradykinin B2 receptor, (23, 40)]; however, it currently is impossible to predict which background will mask or enhance the importance of a gene for blood pressure regulation. Having similar basal blood pressures between genotypes was helpful for the current studies for assessing renal function.

P2Y2 receptor activation can antagonize Na+ reabsorption in the kidney, based on studies in isolated perfused tubule and collecting duct segments and whole animals, as well as patch-clamp experiments (21, 32, 41, 59). However, studies of systemic activation of P2Y2/4 receptors had not been performed. Our use of INS45973 revealed a dose-dependent natriuretic response in WT but not P2Y2−/− mice, while GFR was not changed, indicating a P2Y2 receptor-mediated inhibitory effect on the tubular and/or collecting duct system. We have proposed that the apical ATP/UTP/P2Y2-receptor system in the ASDN has inhibitory effects on ENaC activity (32, 41) and NKCC2 in thick ascending limb (41). Our finding that INS45973 increases fractional excretion of Na+ and K+ via P2Y2 receptor activation may indicate a contribution of NKCC2 inhibition. Changes in blood pressure cannot explain these effects since P2Y2 receptor activation reduced blood pressure which, by itself, is expected to be antinatriuretic.

Recent studies in rats revealed a profound renal effect of naturally occurring diadenosine polyphosphates, e.g., Ap4A, which can activate multiple purinergic receptors, including P2Y2/4 receptors in the kidney. Ap4A possesses natriuretic activity despite a small reduction in GFR and ~10 mmHg decrease in blood pressure (16); suramin inhibits these renal effects (32). Those findings are similar to the effects we observed in WT mice continuously infused with INS45973 (experiment 3). Our studies in P2Y2−/− mice provide evidence that activation of P2Y4 receptors in the kidney may increase reabsorption. Further studies are required to determine the role of renal P2Y4 receptors and whether the blood pressure and renal effects of Ap4A are mediated by P2Y2 receptors.

**Perspectives and Significance**

The current results show that in vivo stimulation of P2Y2 receptors provokes an acute decrease in blood pressure, which is independent of endothelial NO. In contrast, in the absence of P2Y2 receptors, the acute blood pressure increase in response to a P2Y2/4 agonist may reflect P2Y4 receptor-mediated vasoconstriction. Thus, P2Y2 and P2Y4 receptors may functionally antagonize one another in the vasculature and in the regulation of blood pressure. The observed renal responses are consistent with an inhibitory effect of P2Y2 receptor activation on Na+ reabsorption. Dual effects of P2Y2 receptor activation on the vasculature and on renal Na+ reabsorption suggest that P2Y2 receptors may have potential as a therapeutic target in hypertension.

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**DISCLOSURES**

José L. Boyer is an Inspire Pharmaceuticals employee and stock options holder.

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