Simultaneous Activation of p38 MAPK and p42/44 MAPK by ATP Stimulates the K\(^+\) Current \(I_{\text{TREK}}\) in Cardiomyocytes

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Living cells exhibit multiple K\(^+\) channel proteins; among these is the recently reported atypical two-pore domain K\(^+\) channel protein TREK-1. Most K\(^+\) currents are modulated by neurohormones and under various pathological conditions. Here, in rat ventricular cardiomyocytes using the whole-cell patch-clamp technique, we characterize for the first time a native TREK-1-like current \(I_{\text{TREK}}\) that is activated by ATP, a purine agonist applied at a micromolar range. This current is sensitive to arachidonic acid, intracellular acidosis, and various K\(^+\) current inhibitors. Reverse transcription-polymerase chain reaction reveals the presence of a TREK-1-like mRNA in rat cardiomyocytes that shows 93% identity with mouse TREK-1. ATP effects are greatly attenuated in the presence of arachidonic acid or HCO\(^{-}\)\(_3\)-induced intracellular acidosis. Using a series of inhibitors, we further demonstrate that the ATP-induced stimulation of \(I_{\text{TREK}}\) implies the activation of cytosolic phospholipase A\(_2\) and the release of arachidonic acid. These events require the simultaneous involvement of p38 MAPK and p42/44 MAPK, respectively, via a cAMP-dependent protein kinase and a tyrosine kinase pathway, whereas the two MAPKs conjugate to activate a mitogen- and stress-activated protein kinase (MSK-1). Our results thus demonstrate the occurrence of a TREK-1-like current in cardiac cells whose activation by purine agonists implies a dual-MAPK cytosolic pathway.

The exceptional diversity of K\(^+\) currents has physiological significance in the heart, where the various currents underlie distinct phases of action potential repolarization. Recent cloning efforts have identified a large number of pore-forming subunits for K\(^+\) channels. They include the voltage-activated, outward rectifying K\(^+\) channels (Kv families) and the inward rectifying K\(^+\) channels (Kir families) that show a single pore-formation region and six or two transmembrane domains, respectively. More recently, a novel class of K\(^+\) channel subunits (TWIK) that possess two pore-forming regions and four transmembrane domains has been cloned and expressed (1–4). All expressed TWIK-related K\(^+\) channels produce instantaneous and noninactivating currents that do not display a voltage-dependent activation threshold. Some show properties of a background K\(^+\) current, whereas others are activated by free fatty acids and stretch (2, 5) and by intracellular acidosis (6).

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TREK-1 and TBAK-1 or TASK-1 are expressed in the whole heart (1, 7, 8). Whether these proteins carry a physiological ionic current is not yet known in any tissue.

Of the purinergic agonists released during ischemia and other pathological conditions, adenosine has been the most extensively studied. However, under similar conditions, ATP is also released into the extracellular space (9, 10). Extracellular ATP modulates the inward rectifying K\(^+\) currents, I\(_{\text{K1}}\) and I\(_{\text{KAA}}\), and a delayed outward rectifying current in atrial cells (11, 12). Recently, it was postulated that tyrosine phosphorylation is involved in the enhancement of the delayed rectifier K\(^+\) current by ATP in guinea pig ventricular cells (13). Besides activating ionotropic P2X receptors, purinergic stimulation by ATP might involve several of the multiple metabotropic P2Y receptors found in cardiac cells (14). P2Y receptors have been shown to be linked to activation of PKA\(^1\) (15), protein kinase C and MAPK (16, 17), and TK (18). ATP also activates phospholipase A\(_2\) (PLA\(_2\)) in several tissues (19–21). In a variety of cell types, PLA\(_2\) activation occurs as a result of phosphorylation by MAPKs (22, 23). In neutrophils, it was recently reported that inhibitors of p38 MAPK and p42/44 MAPK individually reduced cytosolic PLA\(_2\) (cPLA\(_2\)) activity, whereas their combined blockade caused a total inhibition of cPLA\(_2\) (24). Arachidonic acid (AA), which is released by cPLA\(_2\), during metabolic inhibition or ischemia, activates a K\(^+\) current, I\(_{\text{KAA}}\), in rat neonatal atrial and adult ventricular cells (25, 26). The channel subunit protein underlying I\(_{\text{KAA}}\) is unknown.

We now report a TREK-like K\(^+\) current in isolated rat ventricular cells. This current is activated by extracellular ATP via the release of arachidonic acid after cPLA\(_2\) activation. The purinergic-dependent activation of cPLA\(_2\) requires the simultaneous activation of both p38 MAPK and p42/44 MAPK by a cAMP-dependent protein kinase and a tyrosine kinase-dependent pathway, respectively.

**EXPERIMENTAL PROCEDURES**

**Myocyte Isolation**—Ventricular myocytes were isolated from the heart of urethane-anesthetized (2 g/kg, i.p.) Wistar rats as described previously (27). The heart was first perfused for 5 min at 35 °C with a nominally Ca-free HEPES-buffered solution containing 117 mM NaCl, 5.7 mM KCl, 4.4 mM NaHCO\(_3\), 1.5 mM KH\(_2\)PO\(_4\), 1.7 mM MgCl\(_2\), 21 mM HEPES, 11 mM glucose, 20 mM taurine and then perfused for 40 min with the same solution plus 30 \(\mu\)M Ca\(^{2+}\) and 1.2 mg/ml collagenase (CLS4; Worthington). The heart was then gently dissociated through the bore of a large-tip pipette, followed by two decantations to separate
dead cells. The cells were then suspended in HEPES buffer with 1 mM Ca\(^{2+}\) and 0.5% bovine serum albumin (pH 7.4). The yield of well-stratified, elongated cells was near 80%.

**Voltage-Clamp Recording**—Recording of K\(^+\) currents was performed using the whole-cell patch-clamp technique at room temperature (22 °C ± 2 °C). The pipette resistance was maintained between 0.9 MΩ to 1.1 MΩ. The series resistance (R\(_S\)), membrane capacitance (C\(_M\)), and time constant of membrane capacitance (τ\(_C\)) were determined on voltage-clamped cells according to the equations:

\[
C_M = \tau_C I(E_p - (1 - (L/J))) \quad \text{and} \quad R_S = \tau_C / C_M
\]

where I\(_C\) is the membrane current, I\(_L\) is the current at the end of the 10-ms pulse, and E\(_p\) is the amplitude of the voltage step (2 mV from a holding potential of ~70 mV).

K\(^+\) currents were recorded during 400-ms-long pulses applied between ~130 mV and ±50 mV every 6 s in 10-mV increments from a holding potential of ~50 mV or from a holding potential of ~80 mV in some cases, as specified. Recordings were digitized at 200 μs with a 12-bit analog-to-digital converter acquired and analyzed using pClamp 6 software.

**Solutions and Drugs**—For experiments, a cell aliquot was put in a Petri dish containing the control solution (117 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl\(_2\), 1.7 mM MgCl\(_2\), 10 mM glucose, and 10 mM HEPES; pH was adjusted to 7.4 with NaOH). After achieving whole-cell patch-clamp configuration, the cell was exposed to different extracellular solutions by positioning it at the extremity of one of six capillaries (250 µm inner diameter). Such a setup allowed rapid changes of solution (~1 s). The control solution was supplemented with 50 μM tetrodotoxin and 2 mM CoCl\(_2\) to block Na\(^+\) and Ca\(^{2+}\) currents, respectively. The internal solution contained 120 mM KCl, 6.8 mM MgCl\(_2\), 5 mM Na\(_2\)ATP, 5 mM sodium creatine phosphate, 0.4 mM Na\(_4\)GTP, 11 mM EGTA, 4.7 mM CaCl\(_2\) (120 mM free Ca\(^{2+}\)), and 20 mM HEPES; pH was adjusted with KOH to 7.2; total K\(^+\) 145 mM.

**Intercellular cAMP Assay**—Cells were preincubated for 10 min in the presence of 0.1 mM isobutylmethylxanthine. Batches of 250,000 cardiac cells were incubated for 6 min at 37 °C with 30 μM ATP-S, 40 μM arachidonic acid, or 1 μM isoproterenol with or without preincubation with specific inhibitors. Incubation was stopped by addition of perchloric acid. After centrifugation, the pellet was dissolved in NaOH and used to estimate protein content (28). The supernatant was neutralized by K\(_2\)CO\(_3\), and the perchlorate precipitate was spun down. An aliquot of the supernatant was used for the cAMP assay. A high specific binding of [3H] prostaglandin E\(_1\) was determined by scintillation counting (22).

**Statistical Analysis**—Comparisons of data were made using Student’s t-test, and p < 0.05 was considered statistically significant.

**RESULTS**

**External Application of ATP Increases K\(^+\) Currents in Ventricular Cardiomyocytes**—In rat ventricular myocytes, three distinct K\(^+\) currents are usually recorded. Hyperpolarizing pulses activate the inward rectifier K\(^+\) current (I\(_{in} \text{K}^+\)) sensitive to low (~100 μM) Ba\(^{2+}\) concentrations, whereas depolarizing pulses induce activation of the transient outward K\(^+\) current (I\(_{out} \text{K}^+\)) sensitive to 4-aminopyridine (~3 mM) and of the delayed rectifier K\(^+\) current (I\(_{K} \text{Ca}^+\)) sensitive to tetraethylammonium (~20 mM) (31). The external application of 30 μM ATP induced an increase in both the outward and inward currents that reached steady state after 6 min (Fig. 1A). The ATP-induced outward current exhibited a fast activation with no inactivation during the imposed voltage steps. In parallel experiments performed in the presence of 3 mM 4-aminopyridine, the time to peak was 7.1 ± 1.3 ms at ±50 mV (n = 10). During hyperpolarizing voltage steps to ~130 mV, the ATP-induced inward current was large, peaked at 5.5 ± 0.3 ms (n = 10), and demonstrated fast inactivation before reaching a steady value after about 100 ms. Mean current density/voltage relationships established during the first 20 ms of the applied voltage step at the peak of the inward and outward current demonstrated a significant increase in the inward current only (Fig. 1B). The outward peak current was hardly enhanced as a result of a weak inhibition of I\(_{in} \text{K}^+\). The ATP-induced mean current density/voltage relationship established at the end of the 400-ms voltage pulses exhibited a weak outward-going rectification above ~60 mV and showed inward rectification below this voltage (Fig. 1C). Similar effects were obtained by applying ATP-S (30 μM), or ATP in the presence of theophylline (100 μM), an inhibitor of adenosine-sensitive P1-purinoceptors, whereas the ATP effects were inhibited by suramin, a P2-purinoceptor antagonist (data not shown). Thus, current activation results from ATP activation of P2-purinergic receptors.

ATP activated a muscarinic-like K\(^+\) current in rat ventricular cells (data not shown), as it does in atrial cells (11). In the continued presence of Ba\(^{2+}\), which itself induced a large transient outward current, the further application of ATP was able to induce sustained outward and inward currents, but not the large transient inward current (Fig. 1D). Under these conditions, the ATP-induced current showed a fast activation phase with no inactivation for both depolarizing and hyperpolarizing pulses. Similarly, on cells that have been pretreated with PTX to inactivate the muscarinic cascade, ATP induced...
sustained inward and outward currents (Fig. 1E). Current/voltage relationships of the ATP-induced sustained current in the presence of Ach or after PTX pretreatment show weak inward and outward rectification with similar reversal potential and conductance (Fig. 1F). Consequently, ATP activates both a muscarinic-like and a specific P2-purinergic-induced current in isolated ventricular myocytes of the rat. In the following experiments, the properties of the latter current were analyzed.

**Pharmacological Characterization of the ATP-induced Outward K⁺ Current**—Several compounds known to affect cardiac K⁺ currents were used to characterize the specific ATP-induced sustained K⁺ current. Their effects are quantified here on the sustained outward current elicited at +50 mV (Table I). The ATP-induced outward K⁺ current was enhanced in K⁺-rich solution, insensitive to glibenclamide, a specific inhibitor of the K<sub>ATP</sub> current, and significantly inhibited by tetracain, Gd³⁺, and by 4-aminopyridine, tetraethylammonium, and Ba²⁺ only at high concentrations (Table I). AA is known to trigger an increase in sustained outward K⁺ currents in cardiomyocytes (25, 26, 32) and to activate several two-pore domain K⁺ channels (2). In the presence of AA, ATP had no further significant effect (Fig. 2; Table I). The effects of both ATP and AA were similar in the presence of indomethacin and NDGA, respectively.

**A TREK-1-like Product Is Expressed in Rat Ventricular Cardiomyocytes**—We used RT-PCR to check for the presence of TREK-1 mRNA in rat ventricular cardiomyocytes using gene-specific primers designed to anneal the cDNA of the mouse TREK-1 K⁺ channel. Fig. 3 shows RT-PCR products generated from the total RNA of these cells, as well as a comparison with the RT-PCR products from mouse embryo hearts. The rat ventricular cardiomyocyte products showed 93% identity with TREK-1 (7). Thus, besides a muscarinic-like inward current, ATP activates a sustained K⁺ current whose characteristics point to the recently described acid-sensitive two-pore domain TREK-1 or to a TREK-1-like channel, which we will refer to as I<sub>TREK</sub>.

**ATP Activates cPLA<sub>2</sub>**—To investigate whether I<sub>TREK</sub> activation by ATP would also require AA production after PLA<sub>2</sub>...
Arachidonic acid and acidosis induce an outward $K^+$ current in adult rat cardiomyocytes. AA, mean current density-voltage relationship of AA-induced current ($\bullet$) obtained by subtraction of currents recorded under control conditions and after a 4-min application of 40 $\mu$M AA ($n = 6$). ○, the lack of ATP-induced current under this condition. Inset, current traces recorded at +50 mV under control conditions and after AA and ATP applications (AA; +ATP). B, mean current density-voltage relationship of bicarbonate-induced current (HCO$_3^-$-induced current; △) obtained by subtraction of currents recorded under control conditions and after a 2-min application of 90 mM HCO$_3^-$ substituted for NaCl ($n = 11$). ○, lack of ATP-induced current under this condition. Inset, represented current traces recorded at +50 mV under control conditions and after HCO$_3^-$ and ATP applications (HCO$_3^-$+ATP).

FIG. 2. Arachidonic acid and acidosis induce an outward $K^+$ current in adult rat cardiomyocytes. AA, mean current density-voltage relationship of bicarbonate-induced current (HCO$_3^-$-induced current; △) obtained by subtraction of currents recorded under control conditions and after a 4-min application of 40 $\mu$M AA ($n = 6$). ○, the lack of ATP-induced current under this condition. Inset, current traces recorded at +50 mV under control conditions and after AA and ATP applications (AA; +ATP). B, mean current density-voltage relationship of bicarbonate-induced current (HCO$_3^-$-induced current; △) obtained by subtraction of currents recorded under control conditions and after a 2-min application of 90 mM HCO$_3^-$ substituted for NaCl ($n = 11$). ○, lack of ATP-induced current under this condition. Inset, represented current traces recorded at +50 mV under control conditions and after HCO$_3^-$ and ATP applications (HCO$_3^-$+ATP).

FIG. 3. A TREK-like mRNA is present in isolated cardiomyocytes. Total RNA (2 $\mu$g) extracted from rat isolated ventricular myocytes was reverse-transcribed and subjected to 40 cycles of PCR amplification. cDNA from an embryonic library (mouse embryo; embryonic day 11; 300 ng) was used as a positive control. These results are representative of three similar experiments.

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Table I
Characteristics of the ATP-induced outward $K^+$ current, $I_{\text{TREK}}$

Data are expressed in pA/picoFarad. Number of cells is between 6 and 12, except for control conditions ($n = 36$ cells, 11 hearts). 4-AP, 4-aminopyridine; TEA, tetraethylammonium.

|                  | Control | High $K^+$(5.4 mM) | Gibten (10 $\mu$M) | Tetraena (100 $\mu$M) | Gd$^{3+}$ (100 $\mu$M) | Ba$^{2+}$ (10 $\mu$M) | 4-AP (3 $\mu$M) | TEA (20 $\mu$M) | HCO$_3^-$ (90 $\mu$M) | AA (40 $\mu$M) |
|------------------|---------|--------------------|-------------------|-----------------------|----------------------|-------------------|--------------|---------------|-------------------|-------------|
| Control          | 4.0 ± 0.3 | 6.3 ± 0.4          | 4.0 ± 0.6         | 3.4 ± 0.4             | 8.3 ± 1.5            | 4.8 ± 0.5         | 4.6 ± 1.4    | 1.6 ± 0.1     | 5.3 ± 0.4         | 6.9 ± 0.7    |
| +ATP             | 5.9 ± 0.5 | 9.9 ± 0.9          | 6.1 ± 0.7         | 3.1 ± 0.3             | 7.3 ± 1.3            | 6.0 ± 0.6         | 5.2 ± 1.6    | 2.4 ± 0.1     | 5.1 ± 0.5         | 7.1 ± 0.8    |
| ATP-induced      | 1.9 ± 0.2$^a$ | 3.6 ± 0.4$^a$   | 2.1 ± 0.4$^a$     | -0.3 ± 0.2$^a$        | -1.0 ± 0.4$^b$       | 1.2 ± 0.3$^a$     | 0.7 ± 0.3$^a$ | 0.7 ± 0.1$^a$  | 0.2 ± 0.6$^b$     | 0.2 ± 0.2$^b$ |

$^* p < 0.05$, significantly different relative to individual controls or to the ATP-induced current in the control conditions ($122 \text{nM Ca}^{2+}$, 2.5 mM $K^+_o$, $n = 36$), respectively.
ATP membrane proteins after a 6-min stimulation of rat ventricular cells by ATP activates the recently described acid- and stretch-sensitive two-pore domain K⁺ channel TREK-1 (2, 5, 6) or a channel with similar characteristics in mammalian cardiac myocytes. This current shares many similarities including activation by AA and intracellular acidosis with I\textsubscript{KAA}, initially reported in rat neonatal atrial cardiomyocytes (25, 26). It was also noticed that I\textsubscript{KAA} was reduced in the presence of most inhibitors of one or the other of the MAPK cascades. This is in line with the reduction in sustained outward current found on long-term (15–20-min) ATP application. This late current decrease could result from a secondary ATP-induced inhibitory effect that was emphasized in the presence of GTP\textsubscript{S} added to the pipette solution rather than from current rundown (data not shown). It could be due to a partial reversion of I\textsubscript{KAA} opening by cAMP/PKA activation as reported previously (7) or attributed to a slow inhibition of Kv1.5 channel activity by AA (34).

The MAPK pathway is found ubiquitously in eukaryotic organisms, where it regulates cell proliferation, differentiation, and many other biological functions, some of which follow phosphorylation of cytosolic proteins, whereas, most often, MAPKs are translocated to the nucleus. Our results demonstrate that two MAPKs are required to induce significant I\textsubscript{TREK} activation that occurs as a consequence of AA release. Figure 4, Purinergic stimulation induces cPLA\textsubscript{2} translocation, AA release, and I\textsubscript{TREK}. Top, adult rat cardiomyocytes were stimulated for 6 min by ATP\textsubscript{S} (30 \muM) in the presence or absence of the different inhibitors indicated below. Bar graphs summarize the relative arachidonic acid release and cPLA\textsubscript{2} membrane fraction increase after a 6-min ATP\textsubscript{S} application. Western blots using a cPLA\textsubscript{2} antibody of cell membrane proteins after a 6-min stimulation of rat ventricular cells by ATP\textsubscript{S} (30 \muM) in the presence or absence of SB202190 (5 \muM), U0126 (10 \muM), or Ro318220 (10 \muM), inhibitors of p38 MAPK, p42/44 MAPK, and MSK-1, respectively. Bottom, bar graph summarizes the relative increases in the ATP-induced current recorded using the whole-cell patch-clamp technique under similar experimental conditions.

**DISCUSSION**

The present results demonstrate for the first time the occurrence of a native TREK-1-like current. Furthermore, purinergic stimulation of cardiomyocytes induces this K⁺ current after the activation of cPLA\textsubscript{2} by a dual-MAPK cascade. Like the expressed TREK-1 current activated by AA or acidosis, the ATP-induced current in cardiomyocytes is sustained, demonstrates fast activation and weak outward rectification, and shows specific sensitivity to various K⁺ channel antagonists. In support, RT-PCR products with high identity to the original mouse TREK-1 are generated in the rat ventricular cardiomyocytes. AA activates several TWIK-like currents in \textit{in vitro} expression systems, including TREK-1, which is also specifically activated by intracellular acidosis (6). Our experiments cannot distinguish whether the AA-induced activation is direct or secondary to the lowering of the intracellular pH (33). However, a direct effect of AA is favored. A secondary effect is unlikely under our patch-clamp conditions, where the fast superfusion of the cell should wash away released compounds, and the pipette solution pH is buffered by 20 mM HEPES. Thus, besides inducing a muscarinic-like inward current (11), ATP activates the recently described acid- and stretch-sensitive two-
ular cells (data not shown). The ATP-induced I_{\text{FREK}} activation, cPLA2 translocation, and AA release also require activation of p42/44 MAPK as shown by Western blot and the inhibitory effects of PD98059 and U0126. The PKA pathway inhibitors did not significantly alter p42/44 MAPK activation. These observations might result from the fact that PKA has a dual activator and inhibitory effect on B-Raf and c-Raf-1, respectively, such that the resulting effect on p42/44 MAPK activation could be very moderate (37). Activation of the p42/44 MAPK pathway by G protein-coupled receptors is tyrosine kinase-dependent because tyrosine kinase inhibitors prevent I_{\text{FREK}} activation, cPLA2 translocation, and AA release. Src-family tyrosine kinases, Fyn and Src, have been implicated in G protein-MAPK activation by angiotensin II in cardiac myocytes (38). Furthermore, we reported previously that purinergic stimulation activates Fyn and FAK to phosphorylate the Cl^-/HCO_3^- exchanger in the rat heart and trigger cell acidosis (18). At odds with the significant inhibition of the ATP-induced cPLA2 activation by PTX in the cPLA2-overexpressing Chinese hamster ovary cell line (39), in cardiomyocytes, PTX does not prevent the ATP effects on I_{\text{FREK}} but it does antagonize the effects of acetylcholine on the fast inward current (Fig. 1D). This indicates that a Cl_\text{d6} protein is not involved in this pathway, whose more initial steps remain to be elucidated.

Considerable efforts have been made in recent years to study the mechanism of activation of cPLA2 and the subsequent release of AA. cPLA2 translocation from the cytosol to the membrane is a critical step for access to phospholipids that require Ca^{2+} ions. cPLA2 is known to be active at Ca^{2+} concentrations found in the cytosol of cardiac myocytes (22, 23). Thus, in vivo and during our biochemical experiments, some part of cPLA2 activation might result from an increase in intracellular Ca^{2+} induced by extracellular ATP stimulation (40, 41). However, our electrophysiological experiments performed in the presence of a high EGTA-buffered Ca^{2+}-containing pipette solution demonstrate a reproducible and large increase in I_{\text{FREK}} (much greater than that seen in the absence of Ca^{2+}) and thus imply other regulatory mechanisms. cPLA2 is a physiological target for both p42/44 MAPK and p38 MAPK (22, 24, 42, 43). Under ATP stimulation, both p42/44 MAPK and p38 MAPK are activated (Fig. 5). Each specific MAPK inhibitor did not affect the activation level of the other MAPK but did prevent significant activation of the whole cascade. These observations in cardiomyocytes are in agreement with previous reports that cPLA2 is the substrate for several MAPKs (44, 45). We therefore suggest that in cardiomyocytes, p42/44 MAPK and p38 MAPK act independently and simultaneously to activate cPLA2, AA release, and I_{\text{FREK}}. These effects might be due to each MAPK phosphorylating a different site among the several consensus sites on cPLA2. However, it should rather be considered that dual activation by both p42/44 MAPK and p38 MAPK has been reported for MSK-1, as well as for MAPKAPK5 (46–48). MSK-1 is a widely expressed enzyme that is found in the heart, although with a 12–30-fold lower density in the cytosol than in the nucleus. During our experiments, Ro318220, a specific MSK-1 inhibitor, markedly reduces cPLA2 translocation and I_{\text{FREK}} activation, as did inhibition of each of p42/44 MAPK and p38 MAPK. One might thus consider that MSK-1 integrates phosphorylation by both MAPKs and then activates cPLA2 (Fig. 6).

In conclusion, we first demonstrate the occurrence of a native TREK-1-like current in rat cardiomyocytes. We further elucidate multiple steps leading to TREK-1 activation during ATP application on cardiac cells. The cascade involved dual-MAPK activation, cPLA2 translocation, and AA release. These purinergic-induced effects might result from the stimulation of different purinoreceptors or from that of only the P2Y_2 subtype that is positively coupled to both cAMP and inositol triphosphate production pathway (49). In vivo, these ATP-induced effects will be complemented by the internal acidosis that follows the ATP-induced activation of the Cl^-/HCO_3^- exchanger (18). Both pathways are of importance under various pathological conditions including ischemia, a period during which ATP is released. Activation of I_{\text{FREK}} is one of the numerous alterations induced by ATP that would contribute to electrophysiological disturbances in the ventricular wall and possibly in the nervous system, in which most cells expressed TREK-1 mRNA.

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