

**Andrea Barbuti, Satoshi Ishii, Takao Shimizu, Richard B. Robinson and Steven J. Feinmark**

*Am J Physiol Heart Circ Physiol* 282:2024-2030, 2002. First published Jan 31, 2002;  
doi:10.1152/ajpheart.00956.2001

**You might find this additional information useful...**

---

This article cites 22 articles, 12 of which you can access free at:

<http://ajpheart.physiology.org/cgi/content/full/282/6/H2024#BIBL>

This article has been cited by 6 other HighWire hosted articles, the first 5 are:

**Understanding the cardiac role of K2P channels: A new TASK for electrophysiologists**

F. Charpentier

*Cardiovasc Res*, July 1, 2007; 75 (1): 5-6.

[Full Text] [PDF]

**Neuronal two-pore-domain potassium channels and their regulation by G protein-coupled receptors**

A. Mathie

*J. Physiol.*, January 15, 2007; 578 (2): 377-385.

[Abstract] [Full Text] [PDF]

**Molecular Physiology of Cardiac Repolarization**

J. M. Nerbonne and R. S. Kass

*Physiol Rev*, October 1, 2005; 85 (4): 1205-1253.

[Abstract] [Full Text] [PDF]

**Selective block of the human 2-P domain potassium channel, TASK-3, and the native leak potassium current, IKSO, by zinc**

C. E. Clarke, E. L. Veale, P. J. Green, H. J. Meadows and A. Mathie

*J. Physiol.*, October 1, 2004; 560 (1): 51-62.

[Abstract] [Full Text] [PDF]

**K+-dependent Cerebellar Granule Neuron Apoptosis: ROLE OF TASK LEAK K+ CHANNELS**

I. Lauritzen, M. Zanzouri, E. Honore, F. Duprat, M. U. Ehrengruber, M. Lazdunski and A. J. Patel

*J. Biol. Chem.*, August 22, 2003; 278 (34): 32068-32076.

[Abstract] [Full Text] [PDF]

Updated information and services including high-resolution figures, can be found at:

<http://ajpheart.physiology.org/cgi/content/full/282/6/H2024>

Additional material and information about *AJP - Heart and Circulatory Physiology* can be found at:

<http://www.the-aps.org/publications/ajpheart>

---

This information is current as of March 17, 2010 .

# Block of the background $K^+$ channel TASK-1 contributes to arrhythmogenic effects of platelet-activating factor

ANDREA BARBUTI,<sup>1</sup> SATOSHI ISHII,<sup>2</sup> TAKAO SHIMIZU,<sup>2</sup>  
RICHARD B. ROBINSON,<sup>1</sup> AND STEVEN J. FEINMARK<sup>1</sup>

<sup>1</sup>Center for Molecular Therapeutics, Department of Pharmacology,  
Columbia University, New York, New York 10032; and <sup>2</sup>Department  
of Biochemistry and Molecular Biology, University of Tokyo, Tokyo, 113-003 Japan

Received 2 November 2001; accepted in final form 30 January 2002

**Barbuti, Andrea, Satoshi Ishii, Takao Shimizu, Richard B. Robinson, and Steven J. Feinmark.** Block of the background  $K^+$  channel TASK-1 contributes to arrhythmogenic effects of platelet-activating factor. *Am J Physiol Heart Circ Physiol* 282: H2024–H2030, 2002. First published January 31, 2002; 10.1152/ajpheart.00956.2001.—Platelet-activating factor (PAF), an inflammatory phospholipid, induces ventricular arrhythmia via an unknown ionic mechanism. We can now link PAF-mediated cardiac electrophysiological effects to inhibition of a two-pore domain  $K^+$  channel [TWIK-related acid-sensitive  $K^+$  background channel (TASK-1)]. Superfusion of carbamyl-PAF (C-PAF), a stable analog of PAF, over murine ventricular myocytes causes abnormal automaticity, plateau phase arrest of the action potential, and early afterdepolarizations in paced and quiescent cells from wild-type but not PAF receptor knockout mice. C-PAF-dependent currents are insensitive to  $Cs^+$  and are outwardly rectifying with biophysical properties consistent with a  $K^+$ -selective channel. The current is blocked by TASK-1 inhibitors, including protons,  $Ba^{2+}$ ,  $Zn^{2+}$ , and methanandamide, a stable analog of the endogenous lipid ligand of cannabinoid receptors. In addition, when TASK-1 is expressed in CHO cells that express an endogenous PAF receptor, superfusion of C-PAF decreases the expressed current. Like C-PAF, methanandamide evoked spontaneous activity in quiescent myocytes. C-PAF- and methanandamide-sensitive currents are blocked by a specific protein kinase C (PKC) inhibitor, implying overlapping signaling pathways. In conclusion, C-PAF blocks TASK-1 or a closely related channel, the effect is PKC dependent, and the inhibition alters the electrical activity of myocytes in ways that would be arrhythmogenic in the intact heart.

two-pore domain potassium channels; *Kenk3* ventricular myocytes; inflammatory lipids; mouse

LETHAL ARRHYTHMIAS commonly occur after myocardial ischemia, especially when the ischemic myocardium is reperfused. These arrhythmias are usually initiated by ectopic activity triggered by early (EADs) and delayed afterdepolarizations (DADs) of the membrane potential. One consequence of ischemia and reperfusion is a

rapid migration of polymorphonuclear leukocytes (PMNL) into the infarcted zone. Activated PMNL bind to activated myocytes and release several substances, including oxygen radicals, proteolytic enzymes, and inflammatory lipids that increase the extent of myocardial injury (15). Depletion of circulating neutrophils or treatment with anti-inflammatory drugs effectively limits the size of the infarct zone and the extent of the damage in hearts from several species (15, 20, 22)

Hoffman et al. (4, 5) demonstrated that activation of PMNL bound to isolated canine myocytes dramatically changed the myocyte transmembrane action potential. These changes included prolongation of the action potential duration (APD), EADs, and in some cases arrest during the plateau phase of the action potential. It was also shown that direct superfusion of myocytes with the inflammatory phospholipid platelet-activating factor (PAF) mimicked the action of activated PMNL and that, under similar conditions, PMNL produce significant levels of PAF. Furthermore, incubation of myocytes with the PAF receptor (PAFR) antagonist CV-6209 prevented both PAF- and PMNL-induced changes in myocyte membrane potential. PAF also induces arrhythmias in mice that overexpress the PAFR when the lipid is administered at intravenous doses that have little effect on wild-type (WT) animals (7). These observations suggested that PMNL-derived PAF could induce triggered activity and thus ventricular arrhythmias.

There is considerable confusion regarding the molecular mechanisms by which PAF could alter the electrical activity of the heart. Although PAF binds to a cell surface, G protein-linked receptor and ultimately increases cytosolic  $Ca^{2+}$  levels (17, 19), little is known about the effects of PAF on membrane channels. Wahler et al. (26) showed that subnanomolar concentrations of PAF markedly decreased the inwardly rectifying  $K^+$  channel ( $I_{K1}$ ) in guinea pig ventricular myocytes, whereas Hoffman et al. (5) suggested that depolarizing  $Na^+$  current may play a role in the arrhythmogenic action of PAF.

Address for reprint requests and other correspondence: S. J. Feinmark, Center for Molecular Therapeutics, Dept. of Pharmacology, Columbia Univ., 630 W168th St., New York, NY 10032 (E-mail: sjf1@columbia.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Taking advantage of genetically modified mice in which PAFR have been knocked out [knockout (KO) mice] (6), we tested the ability of carbamyl-PAF (C-PAF), a nonmetabolizable PAF analog, to alter the membrane potential of isolated murine ventricular myocytes with the intent of clarifying the mechanisms determining the arrhythmic effects of this lipid.

## METHODS

**Cell preparation.** Adult mice, 2–3 mo old, were anesthetized with ketamine-xylazine, and their hearts were removed according to protocols approved by the Columbia University Institutional Animal Care and Use Committee. Experiments were performed on single, rod-shaped, quiescent ventricular myocytes dissociated using a standard retrograde collagenase perfusion (11) from hearts of mice that were either WT or PAFR KO. Both WT and KO mice were bred on a C57/Bl6 background. The derivation of the KO mice has been described previously (5).

**Heterologous expression.** The TWIK-related acid-sensitive  $K^+$  background channel (TASK-1) clone (provided by Professor Y. Kurachi, Osaka University; Osaka, Japan) was cotransfected in CHO cells with CD8 plasmid using Lipofectamine Plus (Invitrogen) according to the manufacturer's instructions. Forty-eight hours later, cells were transferred to the electrophysiology chamber, and anti-CD8-coated beads (DynaL Biotech) were added to identify CD8-expressing cells. The CD8-expressing cells were voltage clamped using a ramp clamp (see *Electrophysiological recordings*). CHO cells were used in these experiments in part because they express endogenous PAFR.

**Buffers and drugs.** Before electrophysiological measurements, cells were placed into the perfusion chamber and superfused at room temperature with Tyrode buffer [containing (in mM) 140 NaCl, 5.4 KCl, 1 CaCl<sub>2</sub>, 1 MgCl<sub>2</sub>, 5 HEPES, and 10 glucose; pH 7.4]. The whole cell patch-clamp technique was used with pipettes having resistances of 1.5–3 MΩ [the intracellular solution contained (in mM) 130 aspartic acid, 146 KOH, 10 NaCl, 2 CaCl<sub>2</sub>, 5 EGTA, 10 HEPES, and 2 MgATP; pH 7.2]. Solutions of C-PAF, the PAFR antagonist CV-6209 (BIOMOL), and the protein kinase C (PKC) inhibi-

tor bisindolylmaleimide I (BIM I; Calbiochem) were prepared in water and diluted in Tyrode buffer before use. An inactive analog of BIM I (BIM V; Calbiochem), anandamide, its non-hydrolyzable analog, methanandamide, and an inhibitor of anandamide hydrolysis, arachidonyltrifluoromethyl ketone (ATFK) (BIOMOL), were dissolved in DMSO and then diluted in Tyrode buffer. The final DMSO concentration did not exceed 0.1%. A custom-made fast perfusion device was used to exchange the solution around the cell within 1 s (2).

**Electrophysiological recordings.** Current and voltage protocols were generated using Clampex 7.0 software applied by means of an Axopatch 200B amplifier and a Digidata 1200 interface (Axon Instruments). During voltage clamp, steady-state current traces were acquired at 500 Hz and final filtered at 10 Hz. During current clamp, membrane voltage was acquired at 5 kHz and filtered at 1 kHz. Ramp clamps were conducted by imposing a voltage ramp (14 mV/s) at an acquisition rate of 500 Hz with 1-kHz filtering. Data were analyzed using pCLAMP 8.0 (Axon) and Origin 6.0 (Microcal) and are presented as means  $\pm$  SE. Steady-state current was determined by computer calculation of average current over a time period of at least 5 s. In all experiments, the *n* value indicates the number of myocytes studied and represents pooled data from at least two (voltage clamp) or three (current clamp) animals. Student's *t*-test, one-way ANOVA, and  $\chi^2$ -tests were used; a value of *P* < 0.05 was considered statistically significant. Records have been corrected for the junction potential, which was measured to be 9.8 mV.

## RESULTS

**C-PAF alters the rhythm of paced, WT ventricular myocytes.** Myocytes from WT mice were paced (cycle length, 1,000 ms) and monitored in current-clamp mode to record action potentials. When the APD was stable for 2 min, cells were superfused with 185 nM C-PAF (Fig. 1), a concentration that elicited electrophysiological effects in 9 of 11 cells. C-PAF-evoked responses occurred after a delay ( $94 \pm 21$  s; range, 23–184 s) and typically included abnormal automaticity (Fig. 1; 110 s) leading to a maintained depolariza-

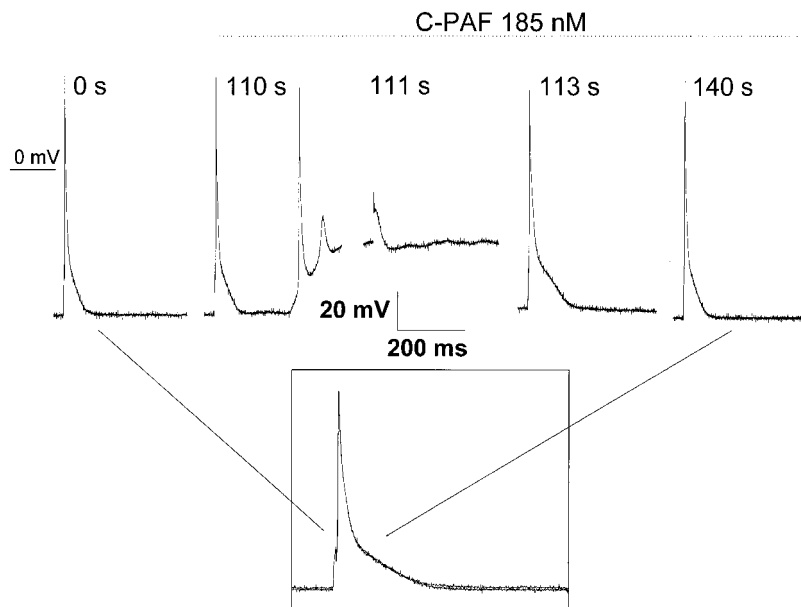


Fig. 1. Carbamyl-platelet-activating factor (C-PAF) alters normal action potentials in mouse ventricular myocytes. Paced action potentials (cycle length, 1,000 ms) were recorded in current-clamp mode under control conditions (0 s) and after perfusion of C-PAF (185 nM). After a delay, C-PAF caused abnormal automaticity (110 s) and sustained depolarization (111 s). The action potential progressively shortened and normal rhythm was reestablished, indicating desensitization of the receptor in continuous presence of drug (113 and 140 s). *Inset:* traces during control perfusion and after recovery completely overlap. The data are derived from a single cell and are typical of 8 cells. The traces were recorded immediately before the application of C-PAF (0 s) and 110, 111, 113, and 140 s after C-PAF application.

tion (Fig. 1; 111 s). In eight of nine cells, alteration of the membrane potential slowly returned to normal, presumably due to receptor desensitization, and after 3 min of agonist perfusion was indistinguishable from that of controls (Fig. 1, *inset*).

*C-PAF* decreases an outward current that is  $K^+$  selective and carried by *TASK-1*. Cells were held at  $-10$  mV, and total steady-state membrane currents were measured. The mean holding current was  $133 \pm 12$  pA ( $n = 24$ ). WT myocytes responded to C-PAF with decreased net outward current that often began to reverse during the perfusion and recovered completely after washout (Fig. 2A). Because a depolarizing shift in steady-state current can be caused by increased inward currents or decreased outward currents, we determined how C-PAF affected conductance. When a  $+10$ -mV step was applied during control and agonist superfusion, we found that C-PAF decreased conductance  $17.5 \pm 3.9\%$  ( $n = 5$ ,  $P < 0.05$ ), indicating that the lipid inhibits outward currents. The main conductance maintaining resting potential in the ventricle is  $I_{K1}$ ; therefore, we tested whether this inwardly rectifying  $K^+$  current was involved in the action of C-PAF.  $Cs^+$  (5 mM), which largely blocks  $I_{K1}$  under these conditions (data not shown), did not reduce the C-PAF-sensitive current in cells held at  $-70$  mV. The average C-PAF-sensitive current density was  $0.047 \pm 0.01$  pA/pF in control cells compared with  $0.047 \pm 0.03$  pA/pF in cells in the presence of  $Cs^+$  ( $n = 6$ ). By extending the voltage-clamp study to other potentials, we obtained a nearly linear current-voltage relation for the C-PAF difference current (Fig. 2B, ■). In KO myocytes, the C-PAF-sensitive current was absent at all potentials tested (Fig. 2B, ●).

We did not observe a clear reversal potential in physiological  $K^+$  over the voltage range tested. Therefore, we conducted additional experiments in elevated extracellular  $K^+$  [50 mM  $K^+$ , with  $Na^+$  reduced to 100 mM, plus 5 mM  $Cs^+$  and 1 mM tetraethylammonium ion ( $TEA^+$ )] designed to measure the reversal potential of the C-PAF-sensitive current. In elevated extracellular  $K^+$ , our results show a weakly outward rectifying current with a current-voltage relation that is consistent with that of a predominantly  $K^+$ -selective channel (Fig. 2C). The calculated  $K^+$  equilibrium potential for these recording conditions is  $-27.6$  mV, and the observed reversal for the C-PAF-sensitive current occurred at  $-20.4 \pm 3$  mV ( $n = 5$ ).

The C-PAF-sensitive current was blocked by the PAFR antagonist CV-6209 (100 nM; Fig. 3). The lack of a C-PAF-dependent response in the presence of CV-6209 was identical to the results obtained in myocytes derived from KO mice (Fig. 3). Taken together, these results confirm that the C-PAF effect is mediated by the PAFR and involves inhibition of an outward  $K^+$  current distinct from  $I_{K1}$ .

These characteristics of the C-PAF-sensitive current suggested that it may be mediated by a member of the "two-pore domain"  $K^+$  channel family (13). *TASK-1* is a member of this family that is expressed in the mammalian heart (9, 10, 13, 14). In heterologous expression

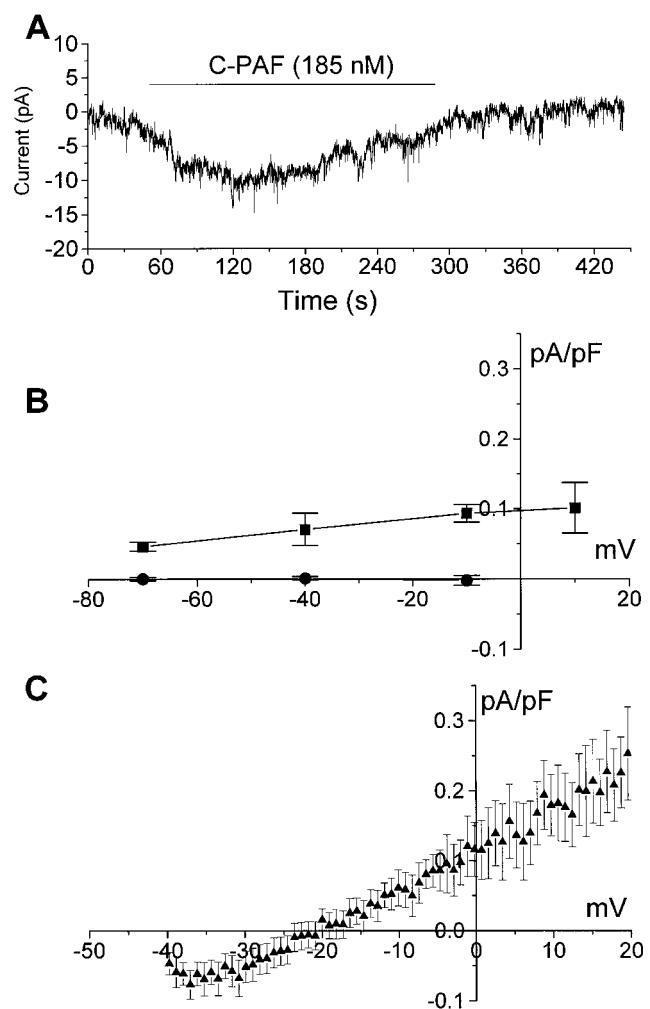


Fig. 2. Application of C-PAF causes a depolarizing shift in net membrane current in wild-type (WT) but not in knockout (KO) myocytes. Superfusion of C-PAF (185 nM) caused a transient decrease in the net outward current in a WT myocyte held at  $-10$  mV (A). In this trace, the baseline outward holding current was adjusted to zero to illustrate the C-PAF-sensitive current. The spontaneous reversal of the C-PAF effect probably indicates desensitization of the PAF receptor (PAFR). The current ( $I$ )-voltage ( $V$ ) relation of the C-PAF difference current (control minus C-PAF) is plotted as a net outward current over a range of potentials in WT myocytes (B, ■). In KO myocytes (●), no C-PAF-sensitive current was detected at all potentials tested. Each data point is the mean  $\pm$  SE of data from at least 4 cells at each potential. The  $I$ - $V$  relation was also measured using a ramp protocol in high extracellular  $K^+$  (50 mM) plus  $Cs^+$  (5 mM) and tetraethylammonium ion (1 mM) to permit determination of the reversal potential (C). Each data point is the mean  $\pm$  SE of data from at least 5 cells from 2 animals.

systems, this channel is outwardly rectifying and is blocked by  $H^+$ ,  $Ba^{2+}$ ,  $Zn^{2+}$ , and anandamide, an endogenous cannabinoid receptor ligand (9, 10, 13, 14, 16, 18, 24). Consistent with this, in isolated myocytes, when the external pH was lowered to 6.4 or when  $Ba^{2+}$  (3 mM) or  $Zn^{2+}$  (3 mM) was present, the C-PAF-sensitive current was significantly reduced (Fig. 4). Methanandamide (10  $\mu$ M), a stable analog of anandamide, also inhibited the C-PAF-sensitive current (Fig. 4). In contrast, anandamide inhibition was only significant in the presence of ATFK (10  $\mu$ M), an inhibitor of

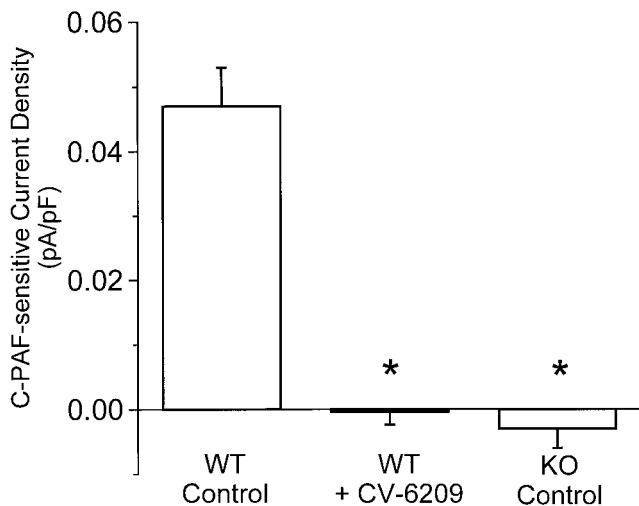


Fig. 3. The C-PAF-sensitive current is receptor mediated. The C-PAF-sensitive current was measured in WT myocytes held at  $-70$  mV under various conditions. The current under control conditions in WT myocytes disappeared in the presence of the PAFR antagonist CV-6209 (100 nM,  $n = 5$ ). There was no C-PAF-sensitive current detected in myocytes from KO mice ( $n = 3$ ). \* $P < 0.01$ .

anandamide hydrolysis (Fig. 4), suggesting rapid metabolism of anandamide by ventricular myocytes. ATFK alone had no effect (data not shown).

CHO cells expressing TASK-1 exhibited a large outwardly rectifying current that was pH sensitive. The mean current-voltage relation at alkaline and acidic

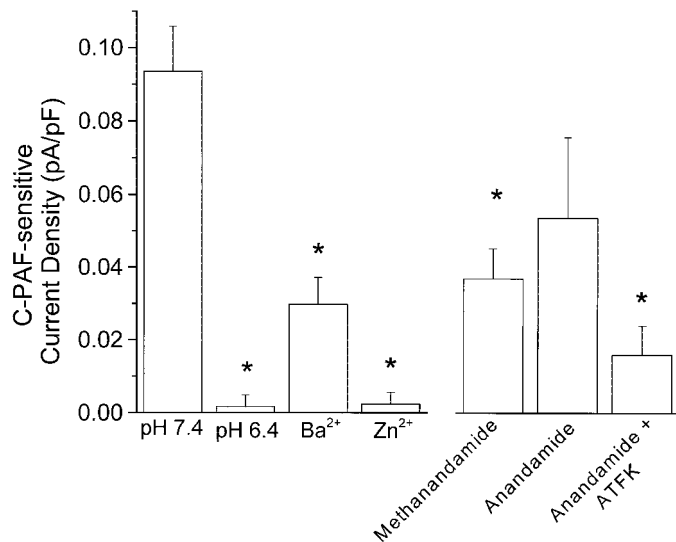


Fig. 4. Block of the TWIK-related acid-sensitive  $K^+$  background channel (TASK-1) decreases the C-PAF-sensitive steady-state current. WT myocytes were held at  $-10$  mV, and the C-PAF-sensitive current was measured at pH 7.4 ( $n = 25$ ). The change in net current elicited by C-PAF (185 nM) was significantly decreased in the presence of Tyrode buffer at pH 6.4 ( $n = 6$ ),  $Ba^{2+}$  (3 mM,  $n = 6$ ), or  $Zn^{2+}$  (3 mM,  $n = 8$ ). The stable anandamide analog methanandamide (10  $\mu$ M,  $n = 12$ ) also significantly reduced the C-PAF-sensitive current, as did anandamide in the presence of arachidonyltrifluoromethyl ketone (ATFK), a drug that inhibits anandamide metabolism (10  $\mu$ M,  $n = 8$ ). Anandamide alone did not significantly inhibit the current (10  $\mu$ M,  $n = 5$ ) due to its rapid metabolic inactivation. \* $P < 0.05$  compared with control at pH 7.4.

pH is shown in Fig. 5, left, and demonstrates that the reduction of the external pH to 6 completely eliminated the outwardly rectifying current. Mean current density at  $+30$  mV in cells expressing TASK-1 was 26 pA/pF compared with 0.6 pA/pF for nontransfected cells. When TASK-1-transfected CHO cells were superfused with C-PAF (185 nM), the expressed current was reduced (Fig. 5, right), demonstrating the inhibitory effect of C-PAF on TASK-1-dependent current.

If both C-PAF and methanandamide block TASK-1, then methanandamide itself should cause a decreased net outward current. Thus the methanandamide-sensitive current was measured (Fig. 6). Because this current is comparable to the C-PAF-sensitive current, we also asked whether the methanandamide-sensitive current was mediated by the PAFR and found that the lipid was fully effective in the presence of the PAFR antagonist CV-6209 or when applied to myocytes from KO mice (Fig. 6). Thus the effect of methanandamide is not mediated by the PAFR.

*C-PAF action involves PKC-dependent block of TASK-1.* In many cell types, PAF initiates an intracellular pathway that results in activation of PKC (1, 17, 19, 23). To determine whether C-PAF initiates this cascade in ventricular myocytes, we incubated cells with BIM I, a selective PKC inhibitor (25) [inhibitory constant, 14 nM], before applying C-PAF. The C-PAF-sensitive current was blocked in a dose-dependent manner (Fig. 7, A and B) by BIM I but was not altered by the addition of an inactive analog, BIM V. The inhibition occurred in a voltage-independent manner (Fig. 7C).

We then asked whether the methanandamide-sensitive current also required PKC activity. BIM I (100 nM) significantly reduced the methanandamide-sensi-

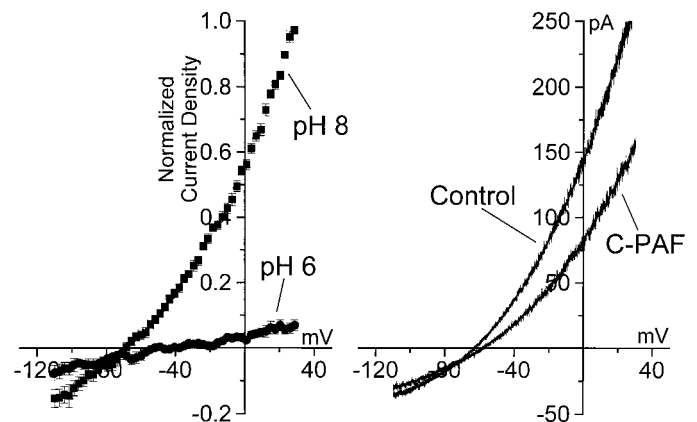


Fig. 5. TASK-1 heterologously expressed in CHO cells is sensitive to pH and to C-PAF. Net steady-state current was measured by a ramp clamp under alkaline (pH 8) and acidic (pH 6) conditions, demonstrating the pH sensitivity of the expressed TASK-1 current. The  $I$ - $V$  relation of each cell was normalized to the current at 30 mV to correct for cell-to-cell variability in expression levels, and the mean normalized current density was plotted (left,  $n = 13$ ). In CHO cells exposed to C-PAF (185 nM), the expressed TASK-1 current was decreased (right). Representative  $I$ - $V$  relations before (control) and during drug treatment (C-PAF) were compared. This result is representative of 8 cells. On average, the  $I$ - $V$  relation returned to within 5% of control value after washout of C-PAF.

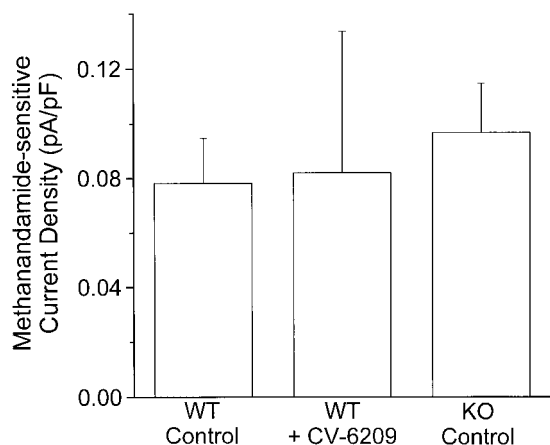


Fig. 6. The methanandamide-sensitive current is independent of the PAFR. WT cells held at  $-10$  mV were superfused with methanandamide ( $10 \mu\text{M}$ ), and the methanandamide-sensitive current was measured (WT control,  $n = 6$ ). The methanandamide-sensitive current did not differ from control when WT cells were incubated with the PAFR antagonist CV-6209 ( $100$  nM,  $n = 3$ ) or in myocytes derived from PAFR knockout mice (KO control,  $n = 6$ ).

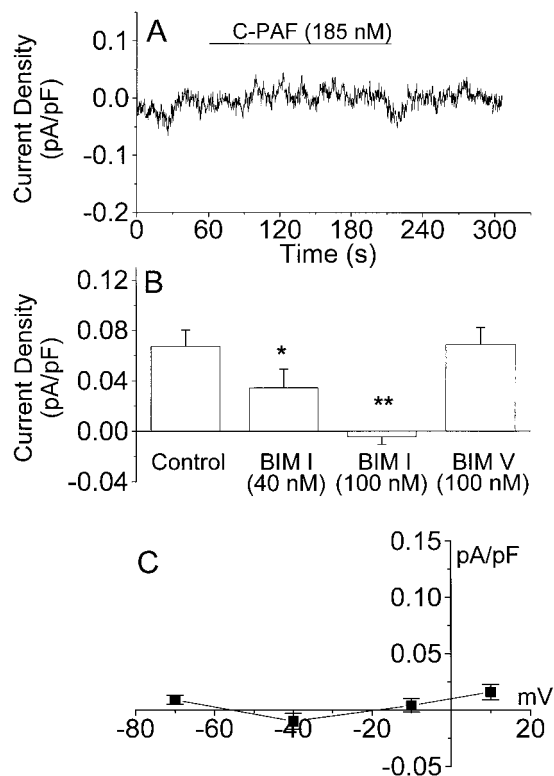


Fig. 7. The C-PAF-sensitive current is blocked by inhibition of protein kinase C (PKC). The C-PAF-sensitive current was completely blocked in myocytes (held at  $-10$  mV) exposed to bisindolylmaleimide I (BIM I), a specific PKC inhibitor ( $100$  nM; A). In this trace, the baseline holding current was adjusted to zero to illustrate the absence of a C-PAF-sensitive current. BIM I-mediated inhibition of the C-PAF-sensitive current is dose dependent (B;  $40$  nM,  $n = 7$ , and  $100$  nM,  $n = 11$ ). An inactive BIM I analog, BIM V, does not block the C-PAF-sensitive current (B;  $n = 10$ ). The inhibition of the C-PAF-sensitive current by BIM I is independent of voltage (C;  $100$  nM BIM,  $n =$  at least 4 for each data point). \* $P < 0.05$  and \*\* $P < 0.001$  vs. control.

tive current in WT myocytes ( $P < 0.05$ ,  $n = 5$ ; data not shown).

*C-PAF and methanandamide induce spontaneous activity in quiescent myocytes.* Because C-PAF and methanandamide affect net steady-state current at voltages near the resting potential, we asked whether electrophysiological effects occurred independent of pacing. Membrane potential was recorded from myocytes that remained quiescent for at least 2 min. Every WT quiescent myocyte tested was sensitive to C-PAF superfusion (11 of 11 cells; Fig. 8A), typically responding with an action potential that arrested in the plateau phase (Fig. 8A, inset), and exhibited many small fluctuations of the membrane potential and EAD. Eventually, the membrane repolarized. The duration of the effect was variable, but its appearance always followed an initial delay ( $96 \pm 11$  s). In contrast, when C-PAF was applied to ventricular myocytes isolated from PAFR KO mice, there was no response in most of the cells (7 of 9 cells;

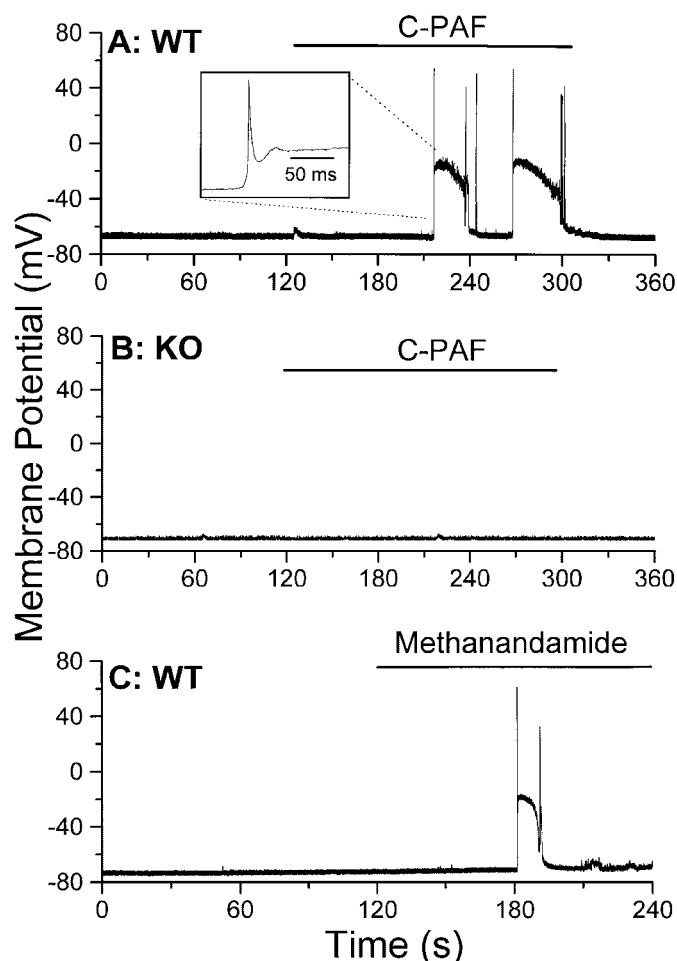


Fig. 8. C-PAF and methanandamide elicit spontaneous activity in quiescent WT myocytes. Quiescent myocytes from WT and KO mice were studied in current-clamp mode. C-PAF ( $185$  nM) application elicited spontaneous activity in WT (A) but not KO myocytes (B). Superfusion of methanandamide ( $10 \mu\text{M}$ ) over WT myocytes caused the same effect as C-PAF (C). There was no measurable change in the resting potential before impulse initiation. These recordings are typical of 11 cells for A, 7 cells for B, and 7 cells for C.

Fig. 8B). The responsiveness of WT and KO myocytes to C-PAF differed significantly ( $P < 0.01$ ,  $\chi^2 = 9.96$ ), although their resting potentials did not ( $-70.6 \pm 1.1$  vs.  $-71.3 \pm 1.5$  mV). Finally, six of eight quiescent WT cells failed to respond to C-PAF (185 nM) after BIM I treatment (100 nM). A comparison of BIM-treated to control myocytes indicated a significant reduction in susceptibility to spontaneous activity ( $P < 0.01$ ,  $\chi^2 = 8.84$ ).

If the decrease in outward current caused by blocking the TASK-1 channel is related to the arrhythmogenic effects of C-PAF, application of a TASK-1 inhibitor in current-clamp mode should mimic the effects of C-PAF and evoke spontaneous activity. Accordingly, when methanandamide was applied to quiescent WT myocytes, spontaneous action potentials were observed (7 of 12 cells; Fig. 8C). Statistical analysis showed no difference in the occurrence of spontaneous activity during methanandamide compared with C-PAF superfusion.

## DISCUSSION

Inflammatory products released by PMNL can have negative effects on cardiac function and the survival of areas at risk after periods of ischemia and reperfusion (15). Our earlier studies in isolated canine ventricular myocytes (4) demonstrated that PAF, a PMNL-derived inflammatory lipid, could alter action potentials by prolongation of the APD, EADs, and arrest at the plateau. The present study demonstrates that in murine ventricular myocytes, C-PAF also triggers a series of alterations in action potentials, including spontaneous beats, EADs, and prolonged depolarization similar to those observed in canine myocytes (4, 5). This supports the validity of the mouse as a model in which to study the molecular basis of the arrhythmogenic effect of PAF.

We measured changes in the membrane potential, spontaneous activity, and in specific ion currents in myocytes as they were exposed to C-PAF. This lipid causes a small change in net current that develops over the first minute after application. Changes in the action potential (or appearance of spontaneous action potentials in quiescent cells) lag behind the peak current by  $\sim 20$  s (at  $-70$  mV, the C-PAF-sensitive current peaked by  $74 \pm 13$  s). The generation of spontaneous activity in quiescent myocytes implies that changes in membrane potential are not strictly dependent on the stimulus or alterations in active currents but rather that it is likely that the agonist perturbs the balance among those currents active at the resting membrane potential. Voltage-clamp experiments measuring changes in conductance indicate that C-PAF effects are dependent on a decrease in outward currents. In addition, the C-PAF-sensitive current, measured in elevated  $K^+$ , showed weak outward rectification and had a reversal potential close to the calculated  $K^+$  equilibrium potential. These data indicate that the C-PAF-sensitive current is largely carried by  $K^+$ .

Because experiments utilizing  $Cs^+$  argue against the involvement of  $I_{K1}$  in the ionic mechanism underlying the PAF-sensitive current, our attention shifted to other  $K^+$  channels that are active at rest. The two-pore domain  $K^+$  channels (13) are voltage- and time-independent background channels having characteristics similar to the channel responsible for the C-PAF-sensitive current. Within this family, TASK-1 [also referred to as cTBAK-1 (9) and Kcnk3 (14)] is expressed in the heart (10). TASK-1 is sensitive to small variations in external pH and is almost completely inhibited at pH 6.4. It is also blocked by  $Ba^{2+}$  or  $Zn^{2+}$  and by the putative endogenous lipid ligand of the cannabinoid receptors anandamide (16). The C-PAF-sensitive current in murine ventricular myocytes was sensitive to all these interventions, suggesting that C-PAF-mediated effects are associated with inhibition of TASK-1 or a closely related channel. Confirmation that the TASK-1 channel is sensitive to C-PAF was obtained by expressing TASK-1 in CHO cells. When TASK-1-expressing CHO cells were superfused with C-PAF, the expressed current was reduced.

Because our data suggested that the C-PAF-sensitive current is due to TASK-1 blockade, we reasoned that anandamide treatment might prevent myocytes from responding to C-PAF. In fact, both anandamide in the presence of ATRF, an inhibitor of anandamide hydrolysis, and its nonhydrolyzable analog, methanandamide, significantly reduced the C-PAF effect, confirming our hypothesis. It follows that if C-PAF and methanandamide both inhibit TASK-1 and if this is the ionic basis for the C-PAF-sensitive effects, methanandamide should induce similar changes in myocyte physiology. As predicted, methanandamide caused both a decrease in net outward current and an increase in spontaneous activity in quiescent myocytes. Therefore, we conclude that both C-PAF and methanandamide exert their biological effects at least in part by inhibiting TASK-1 or a closely related channel.

In a heterologous expression system, Maingret et al. (16) found that anandamide inhibition of TASK-1 was not mediated by the known cannabinoid receptors, and, because the drug was effective on excised macropatches, they concluded that the lipid interacted directly with the channel. PAF, in contrast, is known to activate cells through a G protein-linked receptor that initiates a signaling cascade involving activation of phospholipase C, generating inositol phosphates and elevating intracellular calcium and diacylglycerol, ultimately activating PKC (1, 8, 17, 19). In our studies, the effect of C-PAF is clearly mediated by the PAFR because its activity can be blocked by the antagonist CV-6209 and is absent in myocytes derived from mice in which the PAFR has been genetically deleted. In addition, we found that inhibition of PKC blocked the C-PAF-sensitive current. Although several reports suggest that TASK-1 is insensitive to PKC activators (3, 12), Lopes et al. (14) found that phorbol 12-myristate 13-acetate causes a slowly developing block of TASK-1 current in an oocyte expression system. This further supports our hypothesis that C-PAF activity is

mediated by activation of PKC-dependent phosphorylation, and, although it does not resolve the mechanism behind the somewhat unexpected time course of the effect, it is entirely consistent with our findings.

Interestingly, PKC inhibition also reduced the methanandamide-sensitive current, suggesting that the two lipids share overlapping intracellular signaling pathways. Therefore, we tested whether methanandamide required the PAFR for its activity and found that it was fully functional in the presence of CV-6209 and in myocytes derived from KO mice. These data suggest that the methanandamide effect is dependent, at least in part, on PKC activation. Alternatively, the block of the TASK-1 channel by methanandamide may require a basal phosphorylation of the channel itself or an accessory protein and thus ultimately depends on, but is not mediated by, PKC. Such a scenario was recently described for a similar effect of anandamide on the VR<sub>1</sub> vanilloid receptor, a nonselective cation channel. In this case, activation of the receptor by anandamide was significantly enhanced when the channel had been phosphorylated by PKC, and anandamide itself stimulated PKC (21).

These results suggest, for the first time, a role for the TASK-1 channel in PAF-mediated arrhythmias. However, additional questions remain. While block of TASK-1 channels could contribute to a longer APD and subsequent EADs, this does not preclude additional effects on other currents active during the action potential plateau, including Ca<sup>2+</sup>, Na<sup>+</sup>, and the delayed rectifier currents. In addition, the mechanism by which TASK-1 blockade might lead to initiation of spontaneous activity in a quiescent myocyte is not clear, because no measurable change in membrane potential was observed immediately preceding initiation of activity induced by either C-PAF or methanandamide. Additional mechanisms, either secondary to the block of TASK-1 or independent of this action, may occur after exposure to PAF. The murine model, and its amenability to genetic manipulations, should prove useful in the ultimate resolution of these remaining questions.

The authors thank Irina Voloshyna and Patricia McLaughlin for excellent technical assistance and Dr. Michael R. Rosen for critical comments on the manuscript.

This work was supported by National Heart, Lung, and Blood Institute Grant HL-56140 and by a Servier Strategic Alliance.

## REFERENCES

1. Chao W and Olson MS. Platelet-activating factor: receptors and signal transduction. *Biochem J* 292: 617–629, 1993.
2. DiFrancesco D, Ferroni A, Mazzanti M, and Tromba C. Properties of the hyperpolarizing-activated current (*i<sub>h</sub>*) in cells isolated from the rabbit sino-atrial node. *J Physiol (Lond)* 377: 61–88, 1986.
3. Duprat F, Lesage F, Fink M, Reyes R, Heurteaux C, and Lazdunski M. TASK, a human background K<sup>+</sup> channel to sense external pH variations near physiological pH. *EMBO J* 16: 5464–5471, 1997.
4. Hoffman BF, Feinmark SJ, and Guo SD. Electrophysiologic effects of interactions between activated canine neutrophils and cardiac myocytes. *J Cardiovasc Electrophysiol* 8: 679–687, 1997.
5. Hoffman BF, Guo SD, and Feinmark SJ. Arrhythmias caused by platelet activating factor. *J Cardiovasc Electrophysiol* 7: 120–133, 1996.
6. Ishii S, Kuwaki T, Nagase T, Maki K, Tashiro F, Sunaga S, Cao WH, Kume K, Fukuchi Y, Ikuta K, Miyazaki J, Kumada M, and Shimizu T. Impaired anaphylactic responses with intact sensitivity to endotoxin in mice lacking a platelet-activating factor receptor. *J Exp Med* 187: 1779–1788, 1998.
7. Ishii S, Nagase T, Tashiro F, Ikuta K, Sato S, Waga I, Kume K, Miyazaki J, and Shimizu T. Bronchial hyperreactivity, increased endotoxin lethality and melanocytic tumorigenesis in transgenic mice overexpressing platelet-activating factor receptor. *EMBO J* 16: 133–142, 1997.
8. Ishii S and Shimizu T. Platelet-activating factor (PAF) receptor and genetically engineered PAF receptor mutant mice. *Prog Lipid Res* 39: 41–82, 2000.
9. Kim D, Fujita A, Horio Y, and Kurachi Y. Cloning and functional expression of a novel cardiac two-pore background K<sup>+</sup> channel (cTBAK-1). *Circ Res* 82: 513–518, 1998.
10. Kim Y, Bang H, and Kim D. TBAK-1 and TASK-1, two-pore K<sup>+</sup> channel subunits: kinetic properties and expression in rat heart. *Am J Physiol Heart Circ Physiol* 277: H1669–H1678, 1999.
11. Kuznetsov V, Pak E, Robinson RB, and Steinberg SF.  $\beta_2$ -Adrenergic receptor actions in neonatal and adult rat ventricular myocytes. *Circ Res* 76: 40–52, 1995.
12. Leonoudakis D, Gray AT, Winegar BD, Kindler CH, Harada M, Taylor DM, Chavez RA, Forsayeth JR, and Yost CS. An open rectifier potassium channel with two pore domains in tandem cloned from rat cerebellum. *J Neurosci* 18: 868–877, 1998.
13. Lesage F and Lazdunski M. Molecular and functional properties of two-pore-domain potassium channels. *Am J Physiol Renal Physiol* 279: F793–F801, 2000.
14. Lopes CMB, Gallagher PG, Buck ME, Butler MH, and Goldstein SAN. Proton block and voltage gating are potassium-dependent in the cardiac leak channel Kcnk3. *J Biol Chem* 275: 16969–16978, 2000.
15. Lucchesi BR and Mullane KM. Leukocytes and ischemia-induced myocardial injury. *Annu Rev Pharmacol Toxicol* 26: 201–224, 1986.
16. Maingret F, Patel AJ, Lazdunski M, and Honore E. The endocannabinoid anandamide is a direct and selective blocker of the background K<sup>+</sup> channel TASK-1. *EMBO J* 20: 47–54, 2001.
17. Massey CV, Kohout TA, Gaa ST, Lederer WJ, and Rogers TB. Molecular and cellular actions of platelet-activating factor in rat heart cells. *J Clin Invest* 88: 2106–2116, 1991.
18. Millar JA, Barratt L, Southan AP, Page KM, Fyffe R, Robertson B, and Mathie A. A functional role for the two-pore domain potassium channel TASK-1 in cerebellar granule neurons. *Proc Natl Acad Sci USA* 97: 3614–3618, 2000.
19. Montrucchio G, Alloatti G, and Camussi G. Role of platelet-activating factor in cardiovascular pathophysiology. *Physiol Rev* 80: 1669–1699, 2000.
20. Mullane KM, Read N, Salmon JA, and Moncada S. Role of leukocytes in acute myocardial infarction in anesthetized dogs: relationship to myocardial salvage by anti-inflammatory drugs. *J Pharmacol Exp Ther* 228: 510–522, 1984.
21. Premkumar L and Ahern GP. Induction of vanilloid receptor channel activity by protein kinase C. *Nature* 408: 985–990, 2000.
22. Romson JL, Hook BG, Kunkel SL, Abrams GD, Schork MA, and Lucchesi BR. Reduction of the extent of ischemic myocardial injury by neutrophil depletion in the dog. *Circulation* 67: 1016–1023, 1983.
23. Shukla SD. Platelet-activating factor receptor and signal transduction mechanisms. *FASEB J* 6: 2296–2301, 1992.
24. Talley E, Lei Q, Sirois J, and Bayliss D. TASK-1, a two-pore domain K<sup>+</sup> channel, is modulated by multiple neurotransmitters in motoneurons. *Neuron* 25: 399–410, 2000.
25. Toullec D, Pianetti P, Coste H, Bellevergue P, Grand-Perret T, Ajakane M, Baudet V, Boissin P, Boursier E, and Loriolle F. The bisindolylmaleimide GF 109203X is a potent and selective inhibitor of protein kinase C. *J Biol Chem* 266: 15771–15781, 1991.
26. Wahler GM, Coyle DE, and Sperelakis N. Effects of platelet-activating factor on single potassium channel currents in guinea pig ventricular myocytes. *Mol Cell Biochem* 93: 69–76, 1990.