# Effect of epinephrine on platelet-activating factor–stimulated human vascular smooth muscle cells

**Peter Vadas, MD, PhD, and Boris Perelman, PhD** *Toronto, Ontario, Canada*

**Background: Animal and human data show that platelet-activating factor (PAF) mediates the life-threatening manifestations of anaphylaxis. Although administration of epinephrine is the mainstay of therapy of acute anaphylaxis, the interaction between epinephrine and PAF has not been studied. In particular, the effect of the timing of epinephrine administration on the action of PAF has not been examined.**
**Objective: Using human vascular smooth muscle cells (HVSMCs), we examined the effect of timing of epinephrine addition on the action of PAF.**
**Methods: The effect of epinephrine on PAF-mediated prostaglandin $E_2$ ($PGE_2$) release from human aortic smooth muscle cells was examined. Epinephrine was added at various times before and after PAF stimulation.**
**Results: HVSMCs stimulated with PAF released $PGE_2$ in a concentration- and time-dependent manner. Whereas preincubation of HVSMCs with epinephrine before the addition of PAF suppressed $PGE_2$ release, treatment with epinephrine after PAF stimulation was less effective with time after PAF stimulation. $PGE_2$ release was suppressed by means of preincubation with 8-bromo–cyclic AMP and forskolin.**
**Conclusions: PAF induced $PGE_2$ release from HVSMCs in a concentration- and time-dependent manner, and early addition of epinephrine was essential for the control of PAF-induced $PGE_2$ release. Epinephrine was most effective when administered before stimulation with PAF but was progressively less effective with time after PAF stimulation. (J Allergy Clin Immunol 2012;129:1329-33.)**

***Key words:*** *Epinephrine, platelet-activating factor, platelet-activating factor receptor, human vascular smooth muscle cells, anaphylaxis*

*Abbreviations used*
cAMP: Cyclic AMP
HVSMC: Human vascular smooth muscle cell
PAF: Platelet-activating factor
PAFR: Platelet-activating factor receptor
$PGE_2$: Prostaglandin $E_2$

Anaphylaxis is a systemic allergic reaction with a constellation of symptoms ranging from mild and self-limited to rapidly fatal.[1,2] Anaphylaxis is most commonly triggered by foods, medications, and insect stings. Currently, management of patients at risk for anaphylaxis relies mainly on avoidance of recognized triggers, desensitization (where feasible), and optimal control of relevant comorbid conditions, such as asthma, cardiovascular disease, and mastocytosis.[3] However, avoidance measures are often ineffective, and accidental exposure can result in an acute anaphylactic reaction. The mainstay of therapy of acute anaphylaxis is the use of epinephrine to halt the progression of anaphylaxis and to reverse potentially life-threatening cardiopulmonary manifestations.[2,4]

Early administration of epinephrine effectively reduces morbidity and mortality in human anaphylaxis,[5] whereas delayed administration of epinephrine is associated with increased mortality because epinephrine becomes progressively less effective in reversing anaphylaxis with the passage of time.[6,7] The therapeutic action of epinephrine is quite nonspecific, and the therapeutic window is narrow. As a result, patients and physicians might be reluctant to use epinephrine early in the course of anaphylaxis because of concerns over the real or perceived adverse effects of this drug.

Platelet-activating factor (PAF) is the most potent phospholipid mediator of allergic reactions recognized to date.[8] PAF acts on target cells through a specific platelet-activating factor receptor (PAFR) on the plasma membranes,[9,10] leading to mobilization of intracellular calcium and release of arachidonic acid and prostaglandins, including prostaglandin $E_2$ ($PGE_2$), from vascular smooth muscle cells.[11-14] Signaling through the PAFR can be downregulated by vasoactive agents that activate adenylate cyclase, leading to increased intracellular cyclic AMP (cAMP) levels.[15,16]

Many manifestations of anaphylaxis have been reproduced by injection of exogenous PAF in animals.[17,18] *In vitro* PAF is rapidly released from antigen-stimulated mast cells and basophils and appears to act through a feedback mechanism, causing degranulation of human mast cells with the resultant release of histamine. PAFR antagonists, which inhibit the binding of PAF to the receptor, exert protective effects against anaphylaxis in animal models,[19] and PAFR knockout mice exhibit a lower rate of mortality caused by systemic anaphylaxis than wild-type mice with intact PAFR.[17]

In human anaphylaxis serum PAF levels are significantly increased and correlate directly with the severity of anaphylaxis.[20] These animal and human data support the predominant role of PAF as a central mediator in anaphylactic shock.

On the basis of the foregoing observations, the authors hypothesized that early addition of epinephrine to PAF-stimulated human vascular smooth muscle cells (HVSMCs) would be most effective in modifying target cell activation, whereas delayed administration of epinephrine would be

From the Department of Medicine, Division of Allergy and Clinical Immunology, St Michael's Hospital, University of Toronto.

Supported by grants-in-aid from the National Peanut Board.
Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.
Received for publication June 6, 2011; revised February 19, 2012; accepted for publication February 22, 2012.
Available online March 27, 2012.

Corresponding author: Peter Vadas, MD, PhD, St Michael's Hospital, Division of Allergy and Clinical Immunology, 30 Bond St, Suite 8-161, Toronto, Ontario M5B 1W8, Canada. E-mail: vadasp@smh.ca.

0091-6749/$36.00

doi:10.1016/j.jaci.2012.02.027

**TABLE I.** Effect of epinephrine concentration on $PGE_2$ release by PAF-stimulated HVSMCs*

| | Epinephrine (μmol/L) | | | | |
|---|---|---|---|---|---|
| | 0 | 0.1 | 1.0 | 5.0 | 10.0 |
| 15-min Incubation | 1366 ± 164 | 1531 ± 307 | 1178 ± 202 | 820 ± 150 | 912 ± 193 |
| 30-min Incubation | 2278 ± 430 | 2037 ± 168 | 1489 ± 137 | 893 ± 230 | 1057 ± 159 |

*HVSMCs were preincubated with epinephrine in concentrations ranging from 0.1 to 10 μmol/L for 15 minutes. Smooth muscle cells were incubated for either 15 or 30 minutes with 10 nmol/L PAF, and culture media were assayed for $PGE_2$ release.

progressively less effective, which is analogous to the situation in human anaphylaxis.

## METHODS

### Reagents

PAF, epinephrine, propranolol, 8-bromo-cAMP, forskolin, and fatty acid–free BSA were purchased from Sigma-Aldrich Canada Ltd (Oakville, Ontario, Canada). HVSMCs, medium 231, growth factor, insulin-transferrin solution, and antibiotics were purchased from Cascade Biologics (Portland, Ore). The $PGE_2$ enzyme immunoassay system was purchased from Amersham Biosciences (Baie d'Urfe, Quebec, Canada).

### Cell culture

Aortic HVSMCs (C-007-5C, Cascade Biologics) were plated on tissue-culture flasks and grown to confluence in medium 231 with growth factor at 37°C in a 5% $CO_2$ atmosphere. Cells were replated onto 6-well plates ($250 \times 10^3$ cells per well) and allowed to grow for 24 hours in medium 231 with growth factor. After 24 hours, medium 231 was removed and replaced with quiescent medium, which contains insulin-transferrin solution, vitamin C, 0.1% fatty acid–free BSA, and no growth factor. Cells were incubated for 48 hours in quiescent medium at 37°C in a 5% $CO_2$ atmosphere. All experiments were conducted on cells incubating in quiescent medium at 37°C in a 5% $CO_2$ atmosphere.

### Drug dilution

Drugs were diluted in quiescent medium and added to the cell culture before experiments.

### Effect of PAF on $PGE_2$ release

PAF has been shown to stimulate $PGE_2$ synthesis and release from smooth muscle cells.[12,13] The effects of PAF-mediated $PGE_2$ release were examined. PAF was diluted in quiescent medium and added to HVSMCs in culture (in triplicates) at final concentrations of 0.1, 1, 10, and 100 nmol/L. Cells were coincubated with PAF for 15 or 30 minutes at 37°C in a 5% $CO_2$ atmosphere. HVSMCs growing under the same conditions but without PAF addition were used as controls. $PGE_2$ release was measured in culture medium by using the $PGE_2$ enzyme immunoassay system, according to the manufacturer's instructions. Results are expressed as means ± SDs.

### Relationship between time of epinephrine addition and PAF-induced release of $PGE_2$ by HVSMCs

The effect of epinephrine on suppressing $PGE_2$ release was tested over the concentration range of 0.1 to 10 μmol/L (Table I). A concentration of 5 μmol/L was noted to be optimal. Epinephrine was added to HVSMCs (to a final concentration of 5 μmol/L) before, concomitant with, and after addition of 10 nmol/L PAF. Epinephrine was added at 5 time points, as follows: 15 minutes (−15) or 7.5 minutes (−7.5) before incubation with PAF, simultaneously with PAF (0), or 7.5 minutes (7.5) or 15 minutes (15) after addition of PAF. Cells were incubated at 37°C in a 5% $CO_2$ atmosphere. Culture supernatants were removed 30 minutes after PAF addition and assayed for $PGE_2$ release. Cells treated with 10 nmol/L PAF without epinephrine were used as controls.

### Effect of adenylate cyclase activation and cAMP analog on $PGE_2$ release by PAF-stimulated HVSMCs

Next we examined the effect of forskolin, an agent that activates adenylate cyclase, and of 8-bromo-cAMP, a cell-permeable analog of cAMP, on $PGE_2$ synthesis and release. HVSMCs were preincubated for 15 minutes at 37°C in a 5% $CO_2$ atmosphere with 5 μmol/L epinephrine, 1 mmol/L 8-bromo-cAMP, or 50 μmol/L forskolin. After 15 minutes of preincubation, culture supernatants were removed, and cells were incubated with 10 nmol/L PAF for an additional 15 minutes at 37°C in a 5% $CO_2$ atmosphere and assayed for $PGE_2$ release. Cells treated with 10 nmol/L PAF were used as controls.

### Effect of propranolol on PAF-induced release of $PGE_2$ by HVSMCs

HVSMCs were preincubated for 15 minutes at 37°C in a 5% $CO_2$ atmosphere with propranolol (100 μmol/L) alone, epinephrine (5 μmol/L) alone, or epinephrine (5 μmol/L) plus propranolol (100 μmol/L). After 15 minutes of preincubation, cells were washed with PBS, 10 nmol/L PAF was added in quiescent medium, and cells were incubated for an additional 15 minutes at 37°C in a 5% $CO_2$ atmosphere. Culture supernatants were assayed for $PGE_2$ release. Cells treated with 10 nmol/L PAF were used as controls.

## RESULTS

### Effect of PAF on $PGE_2$ release

HVSMCs stimulated with PAF released $PGE_2$ in a concentration- and time-dependent manner (Fig 1). HVSMCs released 1473 pg/mL $PGE_2$ after 15 minutes of incubation with 100 nmol/L PAF compared with 127 pg/mL $PGE_2$ by the untreated control (11.6-fold increase) and 2172 pg/mL $PGE_2$ after 30 minutes of incubation with 100 nmol/L PAF compared with 127 pg/mL $PGE_2$ by the untreated control (17.1-fold increase).

### Relationship between time of epinephrine addition and PAF-induced release of $PGE_2$ by HVSMCs

Preincubation of HVSMCs with epinephrine before the addition of 10 nmol/L PAF significantly suppressed $PGE_2$ release ($P < .001$, Fig 2). However, coaddition of epinephrine with PAF or addition of epinephrine after PAF stimulation was progressively less effective with time.

### Effect of adenylate cyclase activation and a cAMP analog on $PGE_2$ release by PAF-stimulated HVSMCs

HVSMCs were preincubated with epinephrine, forskolin, and 8-bromo-cAMP (Fig 3). $PGE_2$ release was effectively suppressed by preincubation with epinephrine ($P < .001$), 8-bromo-cAMP ($P < .01$), and forskolin ($P < .001$).

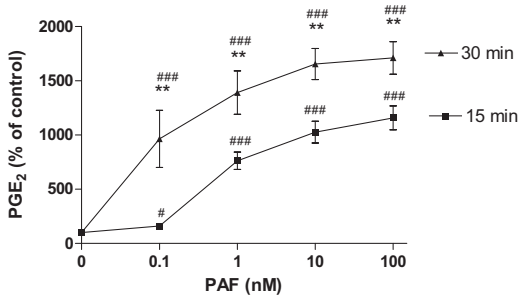


**FIG 1.** Time and dose dependency of PAF-stimulated $PGE_2$ release from HVSMCs. PAF was added to HVSMCs at final concentrations of 0.1, 1, 10, and 100 nmol/L. Cells were coincubated with PAF for 15 or 30 minutes. HVSMCs growing under the same conditions but without PAF addition were used as controls. $PGE_2$ release was measured in culture medium. All experiments were repeated in triplicates. *Solid squares,* $PGE_2$ release from HVSMCs incubated with 100 mmol/L PAF for 15 minutes. *Solid triangles,* $PGE_2$ release from HVSMCs incubated with 100 mmol/L PAF for 30 minutes. $^{**}P < .01$ for 15 minutes versus 30 minutes of incubation with PAF. $\#P < .05$ and $\#\#\#P < .001$ compared with unstimulated controls.

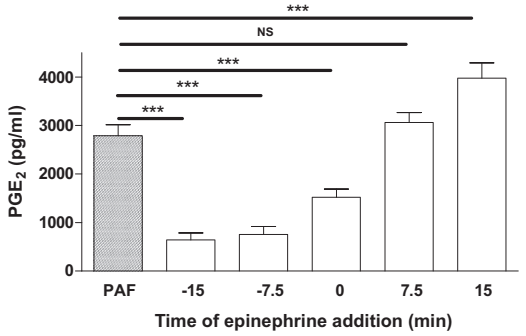


**FIG 2.** Relationship between time of epinephrine addition and PAF-induced $PGE_2$ release. Epinephrine (5 μmol/L) was added to HVSMCs at 15 minutes *(−15)* or 7.5 minutes *(−7.5)* before incubation with 10 nmol/L PAF, simultaneously with PAF *(0)*, or 7.5 minutes *(7.5)* or 15 minutes *(15)* after addition of PAF. Culture supernatants were removed 30 minutes after PAF addition and assayed for $PGE_2$ release. Cells treated with 10 nmol/L PAF without epinephrine were used as controls. $^{***}P < .001$ compared with treatment with PAF alone. *NS,* Not significant. *Bars* represent means ± SDs of triplicate experiments.

## Effect of propranolol on PAF-induced release of $PGE_2$ by HVSMCs

Preincubation of HVSMCs with 5 μmol/L epinephrine before addition of 10 nmol/L PAF suppressed PAF-stimulated $PGE_2$ release by 77% compared with control values. Propranolol reversed the effect of epinephrine on PAF-stimulated $PGE_2$ release (Fig 4).

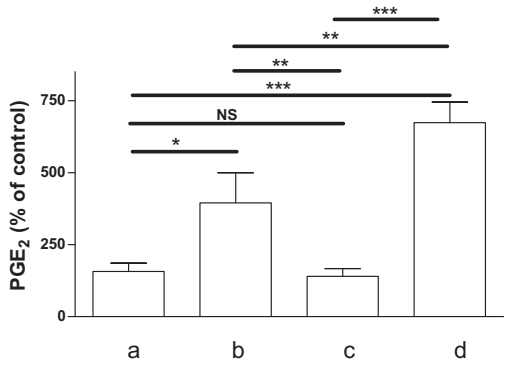


**FIG 3.** Effect of adenylate cyclase activation and a cAMP analog on $PGE_2$ release by PAF-stimulated HVSMCs. HVSMCs were preincubated for 15 minutes with epinephrine, 8-bromo-cAMP, or forskolin. Culture supernatants were removed, and cells were incubated with 10 nmol/L PAF for an additional 15 minutes and assayed for $PGE_2$ release. Cells treated with 10 nmol/L PAF alone served as controls. *a,* Epinephrine (5 μmol/L) and PAF; *b,* 8-bromo-cAMP (1 mmol/L) and PAF; *c,* forskolin (50 μmol/L) and PAF; and *d,* PAF alone. $^{*}P < .05$, $^{**}P < .01$, and $^{***}P < .001$. *NS,* Not significant. *Bars* represent means ± SDs of triplicate experiments.

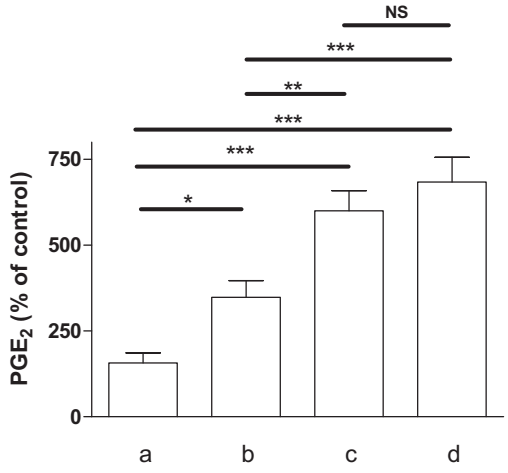


**FIG 4.** Effect of propranolol on PAF-induced release of $PGE_2$ by HVSMCs. HVSMCs were preincubated for 15 minutes with propranolol alone, epinephrine alone, or epinephrine plus propranolol. PAF (10 nmol/L) was added, cells were incubated for an additional 15 minutes, and culture supernatants were assayed for $PGE_2$ release. Cells treated with 10 nmol/L PAF were used as controls. HVSMCs were coincubated with PAF (10 nmol/L) and epinephrine (5 μmol/L; *a*); PAF (10 nmol/L), epinephrine (5 μmol/L), and propranolol (100 μmol/L; *b*); PAF (10 nmol/L) and propranolol (100 μmol/L; *c*); and PAF (10 nmol/L; *d*). $^{*}P < .05$, $^{**}P < .01$, and $^{***}P < .001$. *NS,* Not significant. *Bars* represent means ± SDs of triplicate experiments.

## DISCUSSION

Anaphylaxis is an acute, potentially life-threatening or fatal systemic reaction characterized by multisystem organ involvement as a result of mediators released from mast cells and basophils. Although the skin and respiratory systems are the main organs involved in the early stages of anaphylaxis,[21] dysfunction of the central and peripheral cardiovascular systems often determines the outcome of anaphylactic events.[22] The release of preformed mediators (eg, histamine, tryptase, chymase, and carboxypeptidase A) and newly synthesized lipid mediators (prostaglandin $D_2$; leukotrienes $C_4$, $B_4$, and $E_4$; and PAF) occurs within minutes.[23,24] Cytokines, such as TNF-α and IL-4, IL-6, and IL-13, are released hours after mast cell activation and are thought to have a role in biphasic anaphylaxis.[19]

The mainstay of treatment of acute anaphylaxis is administration of epinephrine in conjunction with other supportive measures.[1,2] Recommendation for early use of epinephrine is currently based on expert opinion and anecdotal evidence. On the other hand, a number of studies have clearly documented the relationship between anaphylactic fatalities and delayed administration of epinephrine.[6,25,26] The mechanism of action of epinephrine is largely unknown,[27] and the therapeutic window is narrow. More specific drugs with better efficacy and

fewer side effects have not yet been developed to treat anaphylaxis.

PAF is the most potent phospholipid mediator of allergic reactions described to date[8] and is biologically active in concentrations as low as $10^{-12}$ mol/L.[18] In spite of its short half-life *in vivo*, PAF can produce sustained manifestations of anaphylaxis. Its biologic effects include increased vascular permeability, decreased cardiac output, circulatory collapse, and smooth muscle contraction in the gut, uterus, and airways.[28] PAF also can potentiate the action of other mast cell products. PAF-like analogs, such as 1-acyl-2-acetyl-sn-glycero-3-phosphocholine, are produced by mast cells, basophils, and endothelial cells. These analogs share many of the same biologic activities as authentic PAF.[29] A variety of cells, including platelets, monocytes, macrophages, eosinophils, Kupfer cells, endothelial cells, and neutrophils,[17,30] have been shown to synthesize and release PAF on stimulation while at the same time exhibiting biologic responses to PAF.[31] Therefore many of the cells that produce PAF also are targets of the bioactivity of PAF. The biologic actions of PAF appear to involve several signaling pathways,[32] such as activation of GTPase activity,[33] G proteins,[32] and phospholipase $A_2$.[34] PAF has been shown to bind to specific PAFRs on the plasma membranes of target cells.[9] Binding studies have revealed 2 distinct types of binding sites on human platelets with high and low affinity for PAF.[35]

PAF acts by binding to a G protein–coupled transmembrane receptor.[32] PAFR is linked through G protein to intracellular signal transduction pathways, including turnover of phosphatidylinositol, increase of intracellular calcium, and activation of several kinases, including inositol 1,4,5-trisphosphate, protein kinase C, and mitogen-activated protein kinase, resulting in the spectrum of biologic activities. It appears that the basic components of this pathway operate in all cells bearing PAFR, whereas the different responses to PAF depend on the function of the target cells. Many signaling events and subsequent functional responses triggered by the PAFR occur in seconds to minutes and do not require new gene expression. However, ligation of PAFR can also lead to nuclear signaling and transcriptional induction of genes involving nuclear factor κB and other transcription factors.[34,36]

Expression of PAFR is regulated by intracellular cAMP levels.[37,38] cAMP downregulates cellular responses to PAF after short exposures, downregulates PAFR mRNA expression after longer exposures,[38,39] and significantly reduces PAF-induced arachidonic acid release.[38] Therefore an increase in intracellular cAMP levels, such as that caused by β-adrenoceptor agonists (eg, epinephrine), represents an important route for controlling PAF activities. On the basis of these data, epinephrine has the potential to modulate the expression of cell-surface PAFRs through an increase in intracellular cyclic AMP levels.

This current study was designed to examine the effect of timing of epinephrine administration on the action of PAF in an *in vitro* model by using HVSMCs to better understand clinical observations regarding the therapeutic use of epinephrine in patients with acute anaphylaxis. This study demonstrated that PAF-induced $PGE_2$ release from HVSMCs in a concentration- and time-dependent manner and that early administration of epinephrine was essential for the control of PAF cell activation, as measured based on PAF-induced $PGE_2$ release. PAF-induced $PGE_2$ release from HVSMCs was downregulated by agents that are known to increase intracellular cAMP levels, including epinephrine, 8-bromo-cAMP, and forskolin. Addition of propranolol to PAF-stimulated HVSMCs reversed the actions of epinephrine, demonstrating that the effect of epinephrine on the regulation of PAF-induced $PGE_2$ release is through β-adrenergic receptors.

*In vitro* murine bone marrow–derived mast cells produced arachidonic acid during FcεRI-mediated activation.[40] Arachidonic acid was converted to $PGE_2$ by microsomal PGE synthase 1 in cocultured fibroblasts, potentially dampening local anaphylactic responses. In contrast, a number of studies have shown that $PGE_2$ might augment anaphylactic reactions. PAF could induce release of $PGE_2$ and other arachidonic acid metabolites from guinea pig lungs in a model of IgG anaphylaxis, suggesting an interaction between PAF and synthesis of eicosanoids.[41] Specific PAFR antagonists dose-dependently decreased the release of $PGE_2$ and other eicosanoids in experimental anaphylaxis.[41-43] $PGE_2$ production was markedly increased locally in anaphylactic lungs and systemically in ovalbumin-induced anaphylactic shock but blocked by treatment with histamine and serotonin receptor blockade in combination with inhibition of nitric oxide synthase.[44] The decreased production of vasodilatory $PGE_2$ was thought to contribute to overall hemodynamic improvement.

Epinephrine presumably modulates intracellular signaling in HVSMCs by increasing intracellular cAMP levels, leading to phosphorylation of the PAFR, although this remains to be studied. There are additional limitations to the current study in that use of an *in vitro* system does not allow for the complex interaction of other mediators relevant to anaphylaxis, particularly under conditions of respiratory alkalosis or metabolic acidosis. Only one read-out of PAF action, namely $PGE_2$, was measured in this study, leaving unknown the effect of epinephrine on the release of other mediators. Arguably, however, the findings of this study are pertinent because of the compelling data showing the centrality of PAF in anaphylaxis in animals and human subjects. Moreover, the progressive reduction in the efficacy of epinephrine with increased time of addition in this *in vitro* system is strikingly similar to the documented reduction in efficacy of epinephrine when its administration is delayed in human anaphylaxis.[5,7,45-47]

The effect of epinephrine on PAF-stimulated HVSMCs is just one aspect of the action of epinephrine and might not be the overriding or most prominent effect. Other recognized mechanisms of action include its $\alpha_1$-adrenergic vasoconstrictor effects in most body organ systems, with the exception of skeletal muscle, and its ability to reverse airway obstruction, mucosal edema, hypotension, and shock.[45,48,49] Other relevant actions in anaphylaxis include its $\beta_1$-adrenergic agonist properties, leading to an increase in the force and rate of myocardial contractility, and its $\beta_2$-adrenergic agonist properties, such as decreased mediator release and relief of bronchospasm.[49] Moreover, these *in vitro* observations do not take into account the pathophysiologic changes as anaphylaxis progresses, such as the reduction in tissue perfusion because of impaired circulation, leading to a corresponding reduction in epinephrine delivery to target organs. Such a reduction in epinephrine delivery would also contribute to its reduced effectiveness as a rescue medication.

This study examined the effect of epinephrine addition on PAF-induced $PGE_2$ release from HVSMCs. Early addition of epinephrine was essential for the control of PAF-induced $PGE_2$ release. Epinephrine was most effective when administered before stimulation with PAF but was progressively less effective with time after PAF stimulation. These observations *in vitro* reflect the observation in human anaphylaxis that early administration of epinephrine is critical for the successful treatment of anaphylaxis.

Further elucidation of the molecular basis for the action of epinephrine will assist in the understanding of the short window of time during which epinephrine is most effective in the treatment of anaphylaxis and in the development of more specific drug therapies for anaphylaxis with fewer unintended side effects.

**Clinical implications: Our findings *in vitro* are consistent with clinical observations showing that epinephrine is most effective when administered early in anaphylaxis and less effective with the passage of time.**

## REFERENCES

1. Sampson HA, Muñoz-Furlong A, Bock SA, Schmitt C, Bass R, Chowdhury BA, et al. Symposium on the Definition and Management of Anaphylaxis: summary report. J Allergy Clin Immunol 2005;115:584-91.
2. Sampson HA, Muñoz-Furlong A, Campbell RL, Adkinson NF Jr, Bock SA, Branum A, et al. Second symposium on the definition and management of anaphylaxis: summary report—Second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network symposium. J Allergy Clin Immunol 2006;117:391-7.
3. Simons FE. Anaphylaxis. J Allergy Clin Immunol 2008;121(suppl):S402-7.
4. Lieberman P. Biphasic anaphylactic reactions. Ann Allergy Asthma Immunol 2005;95:217-26.
5. Pumphrey RS. Lessons for management of anaphylaxis from a study of fatal reactions. Clin Exp Allergy 2000;30:1144-50.
6. Sampson H, Mendelson L, Rosen J. Fatal and near-fatal anaphylactic reactions to food in children and adolescents. N Engl J Med 1992;327:380-4.
7. Pumphrey RS, Gowland MH. Further fatal allergic reactions to food in the United Kingdom 1999-2006. J Allergy Clin Immunol 2007;119:1018-9.
8. Stafforini DM, McIntyre TM, Zimmerman GA, Prescott SM. Platelet-activating factor, a pleiotrophic mediator of physiological and pathological processes. Crit Rev Clin Lab Sci 2003;40:643-72.
9. Honda Z, Nakamura M, Miki I, Minami M, Watanabe T, Seyama Y. Cloning by functional expression of platelet-activating factor receptor from guinea-pig lung. Nature 1991;349:342-6.
10. Izumi T, Shimizu T. Platelet-activating factor receptor: gene expression and signal transduction. Biochim Biophys Acta 1995;1259:317-33.
11. Doyle VM, Creba JA, Ruegg UT. Platelet activating factor mobilizes intracellular calcium in vascular smooth muscle cells. FEBS Lett 1986;197:13-6.
12. Kawaguchi H, Yasuda H. Platelet activating factor stimulates prostaglandin synthesis in cultured cells. Hypertension 1986;8:192-7.
13. Gaumond F, Fortin D, Stankova J, Rola-Pleszczynski M. Differential signaling pathways in platelet activating factor-induced proliferation and interleukin-6 production by rat vascular smooth muscle cells. J Cardiovascular Pharmacol 1997; 30:169-75.
14. Nakao A, Watanabe T, Bitoch H, Imaki H, Suzuki T, Asano K, et al. cAMP mediates homologous downregulation of PAF receptor mRNA expression in mesangial cells. Am J Physiol 1997;273:F445-50.
15. Chao W, Liu H, Zhou W, Hanahan D, Olson M. Regulation of platelet activating factor receptor and platelet activating factor receptor-mediated biological responses by cAMP in rat Kupffer cells. J Biol Chem 1990;265:17576-83.
16. Ashikaga T, Kishi Y, Numano F. Comparison of the effects of vasoactive agents on adenylate cyclase activity in endothelial and smooth muscle cells from the same specimen of fetal bovine aorta. Clin Exp Pharmacol Physiol 1996;23:36-42.
17. Montrucchio G, Alloatti G, Camussi G. Role of platelet activating factor in cardiovascular pathophysiology. Physiol Rev 2000;80:1669-99.
18. Prescott SM, Zimmerman GA, McIntyre TM. Platelet-activating factor. J Biol Chem 1990;265:17381-4.
19. Finkelman FD, Rothenberg ME, Brandt EB, Morris SC, Strait RT. Molecular mechanisms of anaphylaxis: lessons from studies with murine models. J Allergy Clin Immunol 2005;115:449-57.
20. Vadas P, Gold M, Perelman B, Liss GM, lack G, Blyth T, et al. Platelet-activating factor, PAF acetylhydrolase, and severe anaphylaxis. N Engl J Med 2008;358: 28-35.
21. Ishii S, Kuwaki T, Nagase T, Maki K, Tashiro F, Sunaga S, et al. Impaired anaphylactic responses with intact sensitivity to endotoxin in mice lacking a platelet-activating factor receptor. J Exp Med 1998;187:1779-88.
22. Marone G, Bova M, Detoraki A, Onorati AM, Rossi FW, Spadaro G. The human heart as a shock organ in anaphylaxis. Novartis Found Symp 2004;257:133-49.
23. Schwartz LB. Effector cells of anaphylaxis: mast cells and basophils. Novartis Found Symp 2004;257:65-74.
24. Marone G, Triggiani M, de Paulis A. Mast cells and basophils: friends as well as foes in bronchial asthma? Trends Immunol 2005;26:25-31.
25. Sicherer SH, Simons FER. Self-injectable epinephrine for first aid management of anaphylaxis. Pediatrics 2007;119:638-46.
26. Bock S, Munoz-Furlong A, Sampson H. Fatalities due to anaphylactic reactions to foods. J Allergy Clin Immunol 2001;107:191-3.
27. McLean-Tooke AP, Bethune CA, Fay AC, Spickett GP. Adrenaline in the treatment of anaphylaxis: what is the evidence? BMJ 2003;327:1332-5.
28. Imaizumi TA, Stafforini DM, Yamada Y, McIntyre TM, Prescott SM, Zimmerman GA. Platelet-activating factor: a mediator for clinicians. J Intern Med 1995;238:5-20.
29. Heery JM, Kozak M, Stafforini DM, Jones DA, Zimmerman GA, McIntyre TM, et al. Oxidatively modified LDL contains phospholipids with platelet-activating factor-like activity and stimulates the growth of smooth muscle cells. J Clin Invest 1995;96:2322-30.
30. Hwang SB. Specific receptors of platelet-activating factor, receptor heterogeneity, and signal transduction mechanisms. J Lipid Mediat 1990;2:123-58.
31. Chao W, Olson MS. Platelet-activating factor: receptors and signal transduction. Biochem J 1993;292:617-29.
32. Shukla SD. Platelet-activating factor receptor and signal transduction mechanisms. Lipids 1991;26:1028-33.
33. Houslay MD, Bojanic D, Wilson A. Platelet-activating factor and U44069 stimulate GTPase activity in human platelets which is distinct from the guanine nucleotide regulatory. Biochem J 1986;234:737-40.
34. Weyrich AS, McIntyre TM, McEver RP, Prescott SM, Zimmerman GA. Monocyte tethering by P-selectin regulates monocyte chemotactic protein-1 and tumor necrosis factor-alpha secretion. Signal integration and NF-kappaB translocation. J Clin Invest 1995;95:2297-303.
35. Valone FH, Coles E, Reinhold VR, Goetzl EJ. Specific binding of phospholipids platelet-activating factor by human platelets. J Immunol 1982;129:1637-41.
36. Bazan NG, Squinto SP, Braquet P, Panetta T, Marcheselli VL. Platelet-activating factor and polyunsaturated fatty acids in cerebral ischemia or convulsions: intracellular PAF-binding sites and activation of a fos/jun/AP-1 transcriptional signaling system. Lipids 1991;26:1236-42.
37. Thivierge M, Parent JL, Stankova J, Rola-Pleszczynski M. Modulation of human platelet-activating factor receptor gene expression by protein kinase C activation. J Immunol 1996;157:4681-7.
38. Chao W, Liu HL, Zhou WG, Hanahan DJ, Olson MS. Regulation of platelet-activating factor receptor and platelet-activating factor receptor mediated biological responses by cAMP in rat Kupffer cells. J Biol Chem 1990;265: 17576-83.
39. Thivierge M, Alami N, Müller E, de Brum-Fernandes AJ, Rola-Pleszczynski M. Transcriptional modulation of platelet-activating factor receptor gene expression by cAMP. J Biol Chem 1993;268:17457-62.
40. Ueno N, Taketomi Y, Yamamoto K, Hirabayashi T, Kamei D, Kita Y, et al. Analysis of two major intracellular phospholipases A(2) (PLA(2)) in mast cells reveals crucial contribution of cytosolic PLA(2)α, not Ca(2+)-independent PLA(2)β, to lipid mobilization in proximal mast cells and distal fibroblasts. J Biol Chem 2011;286:37249-63.
41. Harczy M, Maclouf J, Pradelles P, Braquet P, Borgeat P, Sirois P. Inhibitory effects of a novel platelet activating factor (PAF) antagonist (BN 52021) on antigen-induced prostaglandin and thromboxane formation by the guinea pig lung. Pharmacol Res Commun 1986;18:111-7.
42. Jancar S, Braquet P, Sirois P. Interactions of arachidonic acid metabolites and platelet activating factor and mechanism of action in hypersensitivity reactions. Braz J Med Biol Res 1987;20:487-94.
43. Sirois P, Harczy M, Braquet P, Borgeat P, Maclouf J, Pradelles P. Inhibition of the release of prostaglandins, thromboxanes, and leukotrienes from anaphylactic lungs by a platelet-activating factor (PAF) antagonist and its stimulation by PAF. Adv Prostaglandin Thromboxane Leukot Res 1987;17:1033-7.
44. Bellou A, Lambert H, Gillois P, Montemont C, Gerard P, Vauthier E, et al. Constitutive nitric oxide synthase inhibition combined with histamine and serotonin receptor blockade improves the initial ovalbumin-induced arterial hypotension but decreases the survival time in brown Norway rats anaphylactic shock. Shock 2003;19:71-8.
45. Simons KJ, Simons FER. Epinephrine and its use in anaphylaxis: current issues. Curr Opin Allergy Clin Immunol 2010;10:354-61.
46. Bock SA, Munoz-Furlong A, Sampson HA. Further fatalities caused by anaphylactic reactions to food, 2001–2006. J Allergy Clin Immunol 2007;119:1016-8.
47. Greenberger PA, Rotskoff BD, Lifschultz B. Fatal anaphylaxis: postmortem findings and associated comorbid diseases. Ann Allergy Asthma Immunol 2007;98:252-7.
48. McLean-Tooke APC, Bethune CA, Fay AC, Spickett GP. Adrenaline in the treatment of anaphylaxis: what is the evidence? BMJ 2003;327:1332-5.
49. Kemp SF, Lockey RF, Simons FER. Epinephrine: the drug of choice for anaphylaxis. A statement of the World Allergy Organization. Allergy 2008;63:1061-70.