**Pasteurella multocida** Toxin Stimulates Mitogen-activated Protein Kinase via $G_{q/11}$-dependent Transactivation of the Epidermal Growth Factor Receptor

(Received for publication, October 4, 1999, and in revised form, November 8, 1999)

Benjamin Seo‡, Eric W. Choy‡, Stuart Maudslay‡, William E. Miller‡, Brenda A. Wilson§, and Louis M. Luttrell¶

From the ‡Department of Medicine, Duke University Medical Center, Durham, North Carolina 27710 and the §Department of Microbiology, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801

The dermonecrotic toxin produced by *Pasteurella multocida* is one of the most potent mitogenic substances known for fibroblasts *in vitro*. Exposure to recombinant *P. multocida* toxin (rPMT) causes phospholipase C-mediated hydrolysis of inositol phospholipids, calcium mobilization, and activation of protein kinase C via a poorly characterized mechanism involving $G_{q/11}$ family heterotrimeric G proteins. To determine whether the regulation of G protein pathways contributes to the mitogenic effects of rPMT, we have examined the mechanism whereby rPMT stimulates the Erk mitogen-activated protein kinase cascade in cultured HEK-293 cells. Treatment with rPMT resulted in a dose and time-dependent increase in Erk 1/2 phosphorylation that paralleled its stimulation of inositol phospholipid hydrolysis. Both rPMT- and $\alpha$-thrombin receptor-stimulated Erk phosphorylation were selectively blocked by cellular expression of two peptide inhibitors of $G_{q/11}$ signaling, the dominant negative mutant G protein-coupled receptor kinase, GRK2(R220R), and the G$_q$ carboxyl-terminal peptide, G$_q$-(305–359). Like $\alpha$-thrombin receptor-mediated Erk activation, the effect of rPMT was insensitive to the protein kinase C inhibitor GP109203X, but was blocked by the epidermal growth factor receptor-specific tyrphostin, AG1478 and by dominant negative mutants of mSos1 and Ha-Ras. These data indicate that rPMT employs $G_{q/11}$ family heterotrimeric G proteins to induce Ras-dependent Erk activation via protein kinase C-independent “transactivation” of the epidermal growth factor receptor.

*Pasteurella multocida* is a wide ranging bacterium found in the respiratory tracts of over 60 avian and 40 mammalian species (1). It is a significant veterinary, and occasional human, pathogen and the cause of osteoclastic bone resorption and severe progressive turbinate damage. The pathogenicity of *P. multocida* is related to the production of a 146-kDa toxin (2, 3) that exhibits little functional homology to other known toxins or proteins. Both native and recombinant PMT are potently mitogenic for several cell types in *in vitro* (4). In Rat1 fibroblasts, rPMT produces anchorage-independent DNA synthesis and growth in soft agar (5). In primary osteoblastic cells in culture, rPMT induces cell proliferation and down-regulation of markers of osteoblast differentiation (6).

The mechanisms whereby rPMT exerts its mitogenic effects are poorly understood. The toxin interacts with a ganglioside-type cell surface receptor and is internalized via both coated and noncoated endocytic structures (7, 8). The mitogenic effects of rPMT require its internalization, since exposure of cells to rPMT at 4 °C or incubation with the weak base methylamine prevents the response (4). Exposure to rPMT stimulates the hydrolysis of inositol phospholipids, calcium mobilization, and phosphorylation of protein kinase C (PKC) substrates, including the myristoylated alanine-rich C kinase substrate protein (4, 5, 9). In addition, rPMT has been shown to stimulate tyrosine phosphorylation of several proteins, including the focal adhesion kinase p125$\text{FAK}$ and paxillin, and to promote both focal adhesion assembly and the formation of actin stress fibers (8, 10).

Several lines of evidence suggest that rPMT exerts at least some of its effects by modulating the activity of $G_{q/11}$ family heterotrimeric G proteins. Treatment of Swiss 3T3 cells with low doses of rPMT strongly potentiates inositol phosphate production following stimulation of $G_{q/11}$-coupled receptors, and rPMT-induced phosphatidylinositol hydrolysis in permeabilized cells is blocked by guanosine 5'-O-(\beta,\gamma-thiotriphosphate) (11). In *Xenopus* oocytes, rPMT-induced calcium-dependent chloride currents are blocked by the injection of specific antisera against PLC$\beta$1 or the $\alpha$ subunit of G$_{q/11}$ and by G$_q$ antisense RNA, and are dramatically enhanced by overexpression of G$_q$ (12).

Recently, many heterotrimeric G protein-coupled receptors (GPCRs) have been shown to activate the Erk mitogen-activated protein kinase pathway. The mechanisms whereby these signals are transduced are characterized by significant heterogeneity. Depending upon cell type, GPCRs have been shown to mediate both Ras-independent Erk activation via stimulation of PKC isoforms, and Ras-dependent Erk activation via activation of receptor and nonreceptor tyrosine protein kinases (13, 14). Since rPMT is thought to mediate many of its effect via the regulation of heterotrimeric G proteins, understanding the mechanisms of PMT-induced mitogenesis might enhance our understanding of the roles of heterotrimeric G proteins in the regulation of cell growth. In this study, we have compared the mechanism of rPMT-mediated Erk activation to that employed by endogenous GPCRs in HEK-293 cells. We find that rPMT 

---

* This work was supported in part by National Institutes of Health Grants DK02352 and DK55524 (to L. M. L.) and AI38396 (to B. A. W.).

† To whom correspondence should be addressed: Dept. of Medicine, Box 3821, Duke University Medical Center, Durham, NC 27710. Tel.: 919-684-2974; Fax: 919-684-8875; E-mail: luttrell@receptor-biol.duke.edu.

‡ The abbreviations used are: PMT, *P. multocida* toxin; rPMT, recombinant *P. multocida* toxin; PKC, protein kinase C; MAP, mitogen-activated protein; GPCR, G protein-coupled receptor; EGF, epidermal growth factor; PMA, phorbol 12-myristate 13-acetate; LPA, lysophosphatidic acid; HA, hemagglutinin; PBS, phosphate-buffered saline.

This paper is available on line at http://www.jbc.org
activates the Erk cascade via the stimulation of Gq11 family G proteins. Subsequently, both rPMT and endogenous Gq11-coupled α-thrombin receptors induce Ras-dependent Erk activation via protein kinase C-independent “transactivation” of the EGF receptor. Thus, rPMT represents a novel bacterial strategy for inducing cell proliferation, by usurping heterotrimeric G protein-regulated mitogenic signals.

EXPERIMENTAL PROCEDURES

Materials—Recombinant P. multocida toxin (rPMT) expressed in Escherichia coli was prepared as described previously (15). Bis-indoylmaleimide I (GP109203X), EGF, and tryptophan AG178 were from Calbiochem. Bordetella pertussis toxin was from List Biologicals. Lysophosphatidic acid (LPA) and phosphor ylmyristate acetate (PMA) were from Sigma. myo-[2-3H]inositol was from NEN Life Science Products. The thrombin agonist hexapeptide H3N-agyr-gy), each with horseradish peroxidase-conjugated anti-rabbit IgG (Jackson Immunoresearch Laboratories), and Gq-(305–359) was detected using a 1:1000 dilution of rabbit polyclonal anti-Gq-(305–359) peptide, derived from the carboxyl-terminal 55 amino acids of Gq.(305–359). The catalytic subunit of Gq/11-coupled G proteins was immunoprecipitated from cells coexpressing putative dominant inhibitory mutant proteins. Following stimulation, monolayers on six-well plates were washed once with PBS and lysed in 1 ml of ice-cold immunoprecipitation precipitation buffer (150 mM NaCl, 50 mM Tris-Cl, pH 8.0, 0.25% w/v sodium deoxycholate, 0.1% v/v Nonidet P-40, 1 mM Na3VO4, 1 mM sodium pyrophosphate, 100 μM Na3VO4, 0.1 mM phenylmethyl-sulfonyl fluoride, 10 μg/ml leupeptin, 10 μg/ml aprotinin). Cell lysates were clarified by centrifugation and immunoprecipitation of HA-Erk1 was performed using 20 μl of HA.11 affinity matrix (Berkeley Antibody Co.) with 2 h agitation at 4°C. Immune complexes were washed twice with ice-cold immunoprecipitation precipitation buffer and once with PBS, denatured in 2× Laemmli sample buffer, and resolved by SDS-polyacrylamide gel electrophoresis. Quantitation of HA-Erk1 phosphorylation was determined by protein immunoblotting using rabbit polyclonal phosphospecific MAP kinase IgG (New England Biolabs) as described.

RESULTS

Recombinant PMT Mediates Erk 1/2 Activation via Pertussis Toxin-insensitive Gq11 Proteins—As shown in Fig. 1, treatment of HEK-293 cells with rPMT resulted in a dose- and time-dependent increase in Erk 1/2 phosphorylation. Erk 1/2 phosphorylation in rPMT-treated cells reached a maximum 6–10-fold over basal increase after 24–48 h of continuous exposure (Fig. 1A). Increasing concentrations of rPMT up to 100 ng/ml produced dose-dependent increases in Erk 1/2 phosphorylation (Fig. 1B), which approximately paralleled rPMT-induced increases in inositol phosphates hydrolysis (Fig. 1C).

Several lines of evidence suggest that the stimulation of inositol phosphate hydrolysis in response to rPMT results from toxin-induced activation of heterotrimeric G proteins (11, 12). To examine whether heterotrimeric G proteins contributed to rPMT-induced activation of the Erk cascade, we initially determined whether rPMT-induced Erk 1/2 activation was additive with that induced by endogenous Gαi and Gq11-coupled receptors. As shown in Fig. 2, stimulation of predominantly Gq11-coupled α-thrombin receptors in HEK-293 cells increased Erk 1/2 phosphorylation 8–10-fold. Pretreatment with rPMT for 24 h prior to stimulation failed to produce an additive response. In contrast, rPMT-mediated Erk 1/2 phosphorylation was additive with that induced by acute stimulation of the predominantly Gq-coupled LPA receptor. Interestingly, the rPMT effect was also not additive with Erk 1/2 phosphorylation induced via the EGF receptor tyrosine kinase.

The lack of additivity between rPMT and α-thrombin receptor-mediated Erk 1/2 activation suggests that a common G protein pool might mediate the response to both stimuli. To determine the role of heterotrimeric G proteins in rPMT-mediated Erk 1/2 activation, we employed three selective inhibitors of G protein function: B. pertussis toxin, which ADP-ribosylates and inactivates Gαi family G proteins, and two inhibitory polypeptides GRK2 (K22020) and Gαi-(305–359). The catalytically inactive mutant of the G protein-coupled receptor kinase (GRK) 2, GRK2(K22020)R, which functions as an dominant negative antagonist of GPCR desensitization (20), has also been shown to directly block Gq11-mediated inositol phosphate production in COS-1 cells (21). This effect results from the direct binding and sequestration of Gq11 subunits by an RGS homology domain in the amino terminus of GRK2 (22). GRK2 also contains a carboxyl-terminal Gβγ subunit binding motif, which inhibits G protein signaling by sequestering free Gβγ subunits (23). The Gαi-(305–359) peptide, derived from the carboxyl-terminal 55 amino acids of Gαi, inhibits Gq11-coupled, but not Gαi-coupled, receptor-mediated PI hydrolysis in COS-7 cells (24).

As shown in Fig. 3, pertussis toxin treatment had no effect on rPMT-stimulated Erk 1/2 phosphorylation in HEK-293 cells. Stimulation of Erk 1/2 by endogenous α-thrombin receptors was minimally affected by pertussis toxin, consistent with Erk
activation mediated predominantly via \(G_{q/11}\) family G proteins. In contrast, LPA-stimulated Erk 1/2 phosphorylation was significantly reduced by pertussis toxin, consistent with a predominantly Gi-mediated response. EGF-stimulated Erk 1/2 activation was pertussis toxin-insensitive.

In contrast to the lack of pertussis toxin effect, cellular expression of GRK2(K220R) and \(G_{q}^{a}(305–359)\) each significantly attenuated rPMT-mediated Erk 1/2 activation. To optimize the detection of signals originating from the transfected cell pool, HEK-293 cells in these experiments were transiently cotransfected with HA epitope-tagged Erk 1 (HA-Erk 1) along with the putative inhibitory peptide, and the phosphorylation state of immunoprecipitated HA-Erk 1 determined. As shown in Fig. 4A, expression of GRK2(K220R) significantly attenuated HA-Erk 1 phosphorylation in response to rPMT treatment, and to stimulation of \(\alpha\)-thrombin and LPA receptors, with no effect on EGF-stimulated HA-Erk 1 activation. Phosphorylation of HA-Erk 1 resulting from cellular expression of a constitutively activated mutant of \(G_{q}^{a}(G_{q}^{a}(Q209L))\) was also attenuated by GRK2(K220R) expression, consistent with direct inhibition of \(G_{q}^{a}\)-dependent signals by GRK2(K220R). Thus, inhibition by GRK2(K220R) is indicative either of a role for...
cDNA encoding HA-Erk 1, plus GαErk 1/2 phosphorylation. HEK-293 cells were transfected with plasmid cDNA encoding Gαq-(305–359) and Gαq(Q209L) as indicated. Left panel shows a protein immunoblot demonstrating expression of Gαq(K220R). Serum-starved transfected cells were incubated for 24 h with rPMT (100 ng/ml) or stimulated for 5 min with SFLLRN (10 μM), LPA (10 μM), or EGF (10 ng/ml) as indicated. Monolayers were lysed in detergent buffer, and the phosphorylation state of immunoprecipitated HA-Erk 1 was determined as described. The mean ± S.E. values for triplicate determinations in one of three independent experiments. B, effect of Gαq-(305–359) expression on Gαq-mediated inositol phosphate production. HEK-293 cells in six-well plates were transfected with plasmid cDNA encoding HA-Erk 1, plus Gαq-(305–359) and Gαq(Q209L) as indicated. Left panel shows a protein immunoblot demonstrating expression of Gαq-(305–359). Transfected cells in serum-free medium were prelabeled with [3H]inositol prior to the determination of basal and SFLLRN-stimulated inositol phosphate production as described. The right panel represents mean ± S.D. values for triplicate determinations in one of three independent experiments. C, effect of Gαq-(305–359) expression on rPMT-stimulated Erk 1/2 phosphorylation. HEK-293 cells were transfected with plasmid cDNA encoding HA-Erk 1, plus Gαq-(305–359) and Gαq(Q209L) as indicated. Serum-starved transfected cells were incubated for 24 h with rPMT (100 ng/ml), or stimulated for 5 min with SFLLRN (10 μM), LPA (10 μM), or EGF (10 ng/ml) as indicated, and the phosphorylation state of immunoprecipitated HA-Erk 1 was determined as described. Upper panel depicts a representative anti-phospho-HA-Erk 1 immunoblot, while the lower panel presents the mean ± S.E. values for three independent experiments.

Collectively, these data suggest that rPMT mediates Erk activation in HEK-293 cells via Gαq11 family G proteins. The sensitivity of sustained rPMT-mediated HA-Erk 1 phosphorylation to GRK2(K220R) and Gαq-(305–359) expression parallels that of the constitutively active Gαq(Q209L) mutant. In addition, the lack of additivity observed between rPMT-mediated Erk activation and that induced by the predominantly Gαq11-coupled α-thrombin receptor suggests that the two signals converge upon a common intermediate.

Recombinant PMT Induces Erk Activation via G Protein-dependent Transactivation of the EGF Receptor Tyrosine Kinase—The mechanisms whereby GPCRs activate the Erk cascade are characterized by extensive heterogeneity (13, 14). Depending upon cell type, receptors coupled to Gα11 proteins have been shown to mediate both Ras-independent Erk activation via stimulation of PKC isoforms, and Ras-dependent Erk activation via activation of receptor and nonreceptor tyrosine protein kinases. In the latter case, GPCR-induced activation of receptor tyrosine kinases, such as the EGF receptor (25, 26), or of focal adhesion kinases (FAK), such as the calcium-dependent FAK kinase Pyk2 (27, 28), serves to initiate a tyrosine phosphorylation cascade leading to Ras activation.

The absence of an additive effect of rPMT treatment on EGF-stimulated Erk 1/2 phosphorylation is consistent with a role for EGF receptors in mediating the rPMT response. In addition, rPMT is known to stimulate both inositol phosphate hydrolysis and to induce calcium- and PKC-independent tyrosine phosphorylation of several proteins, including p125FAK and paxillin (10). Thus, it is plausible that either mechanism could account for rPMT-stimulated Erk activation. To discriminate between these mechanisms, we assessed the sensitivity of rPMT-mediated Erk 1/2 activation to specific PKC and tyrosine kinase inhibitors. As shown in Fig. 5A, inhibition of classical PKC isoforms with GF109203X blocked acute phorbol ester-induced Erk phosphorylation of several proteins, including p125FAK and paxillin (10). Thus, it is plausible that either mechanism could account for rPMT-stimulated Erk activation. To discriminate between these mechanisms, we assessed the sensitivity of rPMT-mediated Erk 1/2 activation to specific PKC and tyrosine kinase inhibitors. As shown in Fig. 5A, inhibition of classical PKC isoforms with GF109203X blocked acute phorbol ester-stimulated Erk phosphorylation, but had no effect on the rPMT, α-thrombin, and EGF receptor-mediated signals. Similar results were obtained using Ro31–8220, a chemically distinct PKC inhibitor with a similar spectrum (data not shown). As shown in Fig. 5B, inhibition of EGF receptor activity using the EGF receptor-specific tyrphostin AG1478 profoundly reduced rPMT, α-thrombin or EGF receptor-mediated Erk phosphorylation, with no effect on the response to phorbol ester. In
incubated with or without tyrphostin AG1478 (100 nM) for 24 h. Cells were either treated with rPMT (100 ng/ml) for 24 h or stimulated for 5 min with SFLLRN (10 μM), EGF (10 ng/ml), or phorbol ester (PMA, 1 μM) prior to the determination of Erk 1/2 phosphorylation. Upper panel depicts a representative immunoblot, while the lower panel presents the mean ± S.E. values for three independent experiments.

FIG. 5. Effect of PKC and EGF receptor inhibitors on Erk 1/2 phosphorylation mediated by rPMT, phorbol ester, and thrombin, LPA, or EGF receptors. A, effect of the protein kinase C inhibitor GF109203X on rPMT-stimulated Erk 1/2 phosphorylation. HEK-293 cells in serum-free medium were incubated with or without GF109203X (2 μM) for 24 h. Cells were either treated with rPMT (100 ng/ml) for 24 h, or stimulated for 5 min with SFLLRN (10 μM), EGF (10 ng/ml), or phorbol ester (PMA, 1 μM) prior to the determination of Erk 1/2 phosphorylation. Upper panel depicts a representative immunoblot, while the lower panel presents the mean ± S.E. values for three independent experiments.

FIG. 6. Effect of dominant negative mutants of mSos1 and Ha-Ras on Erk 1/2 phosphorylation mediated by rPMT, phorbol ester, and thrombin, LPA, or EGF receptors. A, expression of the Sos-PRO and N17 Ras constructs in HEK-293 cells. HEK-293 cells were transfected with plasmid DNA encoding HA-Erk 1, plus Sos-PRO and N17 Ras as indicated. Protein immunoblots of whole cell lysates demonstrate expression of each construct. B, effect of Sos-PRO and N17 Ras expression on rPMT-stimulated Erk 1/2 phosphorylation. Serum-starved transfected cells were incubated for 24 h with rPMT (100 ng/ml), or stimulated for 5 min with SFLLRN (10 μM), LPA (10 μM), EGF (10 ng/ml), or PMA (1 μM) as indicated. Monolayers were lysed in detergent buffer, and the phosphorylation state of immunoprecipitated HA-Erk 1 was determined as described. Left panel depicts a representative anti-phospho-HA-Erk 1 immunoblot, while the right panel presents the mean ± S.E. values for three independent experiments.

**Discussion**

Despite growing evidence that many of the cellular effects of rPMT are mediated by the activation of G(q/11) family heterotrimeric G proteins, the detailed molecular mechanism of rPMT action remains poorly understood. Like other bacterial toxins with intracellular loci of action, binding to cell surface receptors and processing through endocytic vesicles appears to be the rate-limiting step for rPMT action on intact cells. A 3-4-h delay is observed between application of rPMT to intact cells and its maximal effects on inositol phospholipid hydrolysis (9), yet phospholipase Cβ-mediated inositol 1,4,5-trisphosphate and calcium responses occur within seconds when the toxin is directly injected into Xenopus oocytes (12).

Unlike several bacterial toxins that modulate G protein activity, rPMT toxin lacks recognizable enzymatic activity. Microinjection of antisera directed against the amino terminus, but not the carboxyl terminus, of rPMT inhibits its biological activity in Xenopus oocytes (12). Although the full-length protein is required for biological activity on intact cells, the amino-terminal 500 residues of the toxin are active when microinjected (15). This region of PMT shares modest homology with the amino-terminal half of the cytotoxic necrotizing factors 1 and 2 from enteropathogenic E. coli (31, 32), which are thought to target Rho family small GTP-binding proteins (32). In the Xenopus system, the effect of rPMT on calcium-dependent chloride channel activation is markedly enhanced by Gq11 overexpression and specifically inhibited by microinjection of antisera against Gq11 (12). Oocytes injected with rPMT fail to respond to a second challenge, suggesting that rPMT may bind directly to Gq11 subunits and induce GTP exchange. Our results, demonstrating that cellular expression of peptide inhibitors of Gq11 signaling blocks rPMT action in intact cells, are consistent with this model.

The activation of Gq11 proteins by rPMT may be sufficient to
account for its mitogenic effects, as well as its effects on inositol phospholipid turnover and PKC activation. Transient expression of a constitutively active mutant of Goq mimics the effects of rPMT, producing sustained increases in phosphatidylinositol hydrolysis and Erk activation that are blocked by peptide inhibitors of Goq11 signaling. The mechanism of rPMT-stimulated Erk activation in HEK-293 cells parallels that employed by the predominantly Goq11-coupled α-thrombin receptor. Both signals are independent of PKC, but dependent upon the catalytic activity of endogenous EGF receptors to activate Ras-dependent pathways.

Both rPMT exposure and acute stimulation of Goq11-coupled receptors induce the tyrosine phosphorylation of multiple substrates. In Swiss 3T3 cells, rPMT stimulates tyrosine phosphorylation of α125FAK and paxillin in a cytochalasin D-sensitive manner (10). Similar results have been obtained following acute stimulation of Goq11-coupled receptors in Swiss 3T3 (33), Rat 1 (34), and HEK-293 cells (35). Our data indicate that rPMT also mediates growth stimulatory effects via G protein-dependent activation of EGF receptors. Indeed, previous work has demonstrated that rPMT exposure promotes the loss of cell surface EGF receptors (36), an effect that may represent EGF receptor down-regulation following rPMT-induced transactivation.

Goq11-coupled receptors have been shown to mediate two, apparently distinct, calcium-dependent tyrosine phosphorylation cascades that converge on Ras and its downstream effectors. In several cell types, GPCR-stimulated Erk activation involves the ligand-independent transactivation of receptor tyrosine kinases, such as the EGF receptor (25, 26). In PC12 cells, bradykinin (37) and m1 muscarinic acetylcholine (38) receptors activate EGF receptors via either calcium- or PKC-dependent mechanisms, respectively. In vascular smooth muscle, angiotensin II mediates calcium-dependent EGF receptor transactivation upstream of the Erk cascade (39). Alternatively, bradykinin-dependent Erk activation has been attributed to stimulation of the calcium-dependent FAK family kinase, Pyk2 (27, 28). Pyk2-dependent signals require either calcium or PKC (27) and are sensitive to cytochalasin D, suggesting a requirement for intact focal adhesions (40). Activated Pyk2, like α125FAK, associates with c-Src and provides docking sites for Grb2-Sos complexes (28).

Despite similarities in their mechanisms of activation (41), Pyk2-mediated signals appear to be independent of those mediated via EGF receptor transactivation. In PC12 cells, expression of a dominant negative mutant of Pyk2 does not inhibit bradykinin-induced transactivation of the EGF receptor, and expression of a dominant negative EGF receptor mutant has no effect on Pyk2 phosphorylation (42). In HEK-293 cells, treatment with tyrphostin AG1478 (35) and expression of a dominant negative mutant of Pyk2 (43) each cause a partial blockade of Goq11-coupled receptor-mediated Erk activation, suggesting that both transactivated EGF receptors and Pyk2 contribute independently to Erk activation. While we find that rPMT-stimulated Erk activation is predominantly tyrphostin-sensitive, we cannot exclude a contribution from Pyk2 acting via a focal adhesion-based scaffold.

Study of the mechanisms of action of bacterial toxins has led to significant insights into the roles of heterotrimeric G proteins in cellular signaling. Our data suggest that rPMT employs Goq11 proteins in a novel strategy for mitogenic regulation by a bacterial toxin, that of utilizing G proteins to initiate a tyrosine phosphorylation cascade leading to Ras activation. Given the potent mitogenic effects achieved by rPMT, these findings serve to underscore the importance of G protein-catalyzed mitogenic signals to growth regulation.

Acknowledgments—We thank R. Lefkowitz for helpful discussion and critical reading of the manuscript. We thank D. Addison and M. Holben for excellent secretarial assistance.

REFERENCES
1. Carter, G. R. (1967) Adv. Vet. Sci. 11, 321–379
2. Buys, W. C. E. M., Smith, H. E., Camps, A. M. I. E., Kamp, E. M., and Smits, M. A. (1990) Nucleic Acids Res. 18, 2815–2819
3. Lax, A. J., Chanter, N., Pullinger, G. D., Higgins, T., Staddon, J. M., and Rozengurt, E. (1990) FEBS Lett. 277, 59–64
4. Rozengurt, E., Higgins, T., Chanter, N., Lax, A. J., and Staddon, J. M. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 123–127
5. Higgins, T., Murphy, A. C., Staddon, J. M., Lax, A. J., and Rozengurt, E. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 4240–4244
6. Mullane, P. H., and Lax, A. J. (1996) Infect. Immun. 64, 959–965
7. Pettit, R. K., Ackermann, M. R., and Rimler, R. B. (1993) Lab. Invest. 69, 94–100
8. Doerner, E. T., Chailier, P., Dubreuil, J. D., and Martinez-Doize, B. (1996) Infect. Immun. 66, 5636–5642
9. Staddon, J. M., Barker, C. J., Murphy, A. C., Chanter, N., Lax, A. J., Michell, R. H., and Rozengurt, E. (1991) J. Biol. Chem. 266, 4840–4847
10. Lax, A. J., Luttrell, L. M., Hawes, B. E., and Lefkowitz, R. J. (1996) Endocrinology 17, 689–714
11. Koch, W. J. (1998) J. Biol. Chem. 273, 1839–1842
12. Wilson, B. A., Ponferrada, V. G., Vallance, J. E., and Ho, M. (1999) Infect. Immun. 67, 80–87
13. Meloche, S., Pages, G., and Pouyssegur, J. (1990) Mol. Biol. Cell 3, 63–71
14. Luttrell, L. M., Ostrowski, J., Cotecechie, S., Kendall, H., and Lefkowitz, R. J. (1993) Science 259, 1453–1457
15. Attramadal, H., Arriza, J. L., Aoki, C., Dawson, T. M., Codina, J., Kwastra, M. M., Snyder, S. H., Carson, M. G., and Lefkowitz, R. J. (1992) J. Biol. Chem. 267, 12768–12775
16. van Biesen, T., Luttrell, L. M., Hawes, B. E., and Lefkowitz, R. J. (1996) Endocr. Rev. 17, 689–714
17. Wilson, W. A., and Staddon, J. M. (1990) Proc. Natl. Acad. Sci. U. S. A. 89, 5476–5481
18. Siderovski, D. P., Strockbine, B., and Behe, C. I. (1999) Crit. Rev. Biochem. Mol. Biol. 34, 215–251
19. Koch, W. J., Hawes, B. E., Ingles, J., Luttrell, L. M., and Lefkowitz, R. J. (1994) J. Biol. Chem. 269, 6193–6197
20. Akhter, S., Luttrell, L. M., Rockman, H. A., Lecocq, G., Lefkowitz, R., and Koch, W. J. (1998) Science 280, 574–577
21. Daub, H., Weiss, F. U., Wallasch, C., and Ullrich, A. (1996) Nature 379, 557–560
22. Daub, H., Wallasch, C., Lankena, A., Herrlich, A., and Ullrich, A. (1997) EMBO J. 16, 7032–7044
27. Lev, S., Moreno, H., Martinez, R., Canoll, P., Peles, E., Mussachio, J. M., Plowman, G. D., Rudy, B., and Schlessinger, J. (1995) *Nature* **376**, 737–745
28. Dikic, I., Tokiwa, G., Lev, S., Courtneidge, S. A., and Schlessinger, J. (1996) *Nature* **383**, 547–550
29. Hawes, B. E., van Biesen, T., Koch, W. J., Luttrell, L. M., and Lefkowitz, R. J. (1995) *J. Biol. Chem.* **270**, 17148–17153
30. van Biesen, T., Hawes, B. E., Luttrell, D. K., Krueger, K. M., Touhara, K., Perfiri, E., Sakaue, M., Luttrell, L. M., and Lefkowitz, R. J. (1995) *Nature* **376**, 781–784
31. Falbo, V., Pace, T., Picci, E., Pixzi, E., and Caprioli, A. (1993) *Infect. Immun.* **61**, 4909–4914
32. Oswald, E., Sugai, M., Labigne, A., Wu, H. C., Fiorentini, C., Boquet, P., and O'Brien, A. D. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91**, 3814–3818
33. Rodriguez-Fernandez, J. L., and Rozengurt, E. (1996) *J. Biol. Chem.* **271**, 27895–27901
34. Luttrell, L. M., Daaka, Y., Della Rocca, G. J., and Lefkowitz, R. J. (1997) *J. Biol. Chem.* **272**, 31648–31656
35. Della Rocca, G. J., Maudeley, S., Daaka, Y., Lefkowitz, R. J., and Luttrell, L. M. (1999) *J. Biol. Chem.* **274**, 13978–13984
36. Staddon, J. M., Chanter, N., Lax, A. J., Higgins, T. E., and Rozengurt, E. (1996) *J. Biol. Chem.* **265**, 11841–11848
37. Zwick, E., Daub, H, Aoki, N., Yamaguchi-Aoki, Y., Tinhofer, I., Maly, K., and Ullrich, A. (1997) *J. Biol. Chem.* **272**, 24767–24770
38. Tsai, W., Morielli, A. D., and Peralta, E. G. (1997) *EMBO J.* **16**, 4597–4605
39. Eguchi, S., Numaguchi, K., Iwasaki, H., M. T., Yamakawa, T., Utsunomiya, H., Motley, E. D., Kawakatsu, H., Owada, K. M., Hirata, Y., Marumo, F., and Inagami, T. (1998) *J. Biol. Chem.* **273**, 8890–8896
40. Li, X., Hunter, D., Morris, J., Haskell, J. S., and Earp, H. S. (1998) *J. Biol. Chem.* **273**, 9361–9364
41. Eguchi, S., Iwasaki, H., Inagami, T., Numaguchi, K., Yamakawa, T., Motley, E. D., Owada, K. M., Marumo, F., and Hirata, Y. (1999) *Hypertension* **33**, 201–206
42. Zwick, E., Wallasch, C., Daub, H., and Ullrich, A. (1999) *J. Biol. Chem.* **274**, 20989–20996
43. Della Rocca, G. J., van Biesen, T., Daaka, Y., Luttrell, D. K., Luttrell, L. M., and Lefkowitz, R. J. (1997) *J. Biol. Chem.* **272**, 19125–19132