

## m3 Muscarinic Acetylcholine Receptor Regulation in the Airway

Charlotte K. Billington and Raymond B. Penn

Department of Microbiology and Immunology, Kimmel Cancer Institute, Thomas Jefferson University, Philadelphia, Pennsylvania

The m3 muscarinic acetylcholine receptor (m3 mAChR) plays an important role in airway function by mediating the effects of acetylcholine on multiple airway cell types. m3 mAChRs expressed in airway smooth muscle (ASM) cells promote increased ASM tension, and, therefore, airway narrowing, in response to acetylcholine release from postganglionic parasympathetic nerves innervating the airway (1). In addition, m3 mAChRs have been implicated in the regulation of mucous secretion in submucosal glands (2) and in chemotactic mediator release in alveolar macrophages (3). Thus, multiple cellular functions that impact resistance to airflow are under the control of m3 mAChRs. Accordingly, insight into the mechanisms by which m3 mAChRs are activated and regulated in the airway are of potential value in understanding obstructive airway diseases and their effective management.

In 1987 two groups, working independently, cloned the cDNA encoding the m3 mAChR (4, 5). As was the case with the cloning of other G protein-coupled receptors (GPCRs), this facilitated extensive investigation into the pharmacology, signaling, and regulation of the receptor by enabling its heterologous expression in various cell culture systems. Although the m3 mAChR has the capacity to activate multiple signaling pathways in various cell types (6–9), activation of phospholipase C (PLC) via the intermediary heterotrimeric G protein Gq is the predominant pathway through which the m3 mAChR regulates important airway cell functions such as ASM contraction (10, 11) (Figure 1). PLC activation induces protein kinase C (PKC) activation and inositol 1,4,5-trisphosphate (IP<sub>3</sub>) generation, which serve to increase intracellular Ca<sup>2+</sup> and sensitize and activate the cell's contractile machinery (12, 13). This basic signaling paradigm is also common to other GPCRs (e.g., the H1 histamine and cysteinyl leukotriene-1 receptors) whose stimulation also induces ASM contraction.

m3 mAChR-mediated transmembrane signaling has been shown to be a highly regulated process. Regulation of the activities of Gq and PLC, via either their phosphorylation,

subcellular localization, or changes in expression levels, has been shown to affect signaling via the m3 mAChR and other Gq-coupled receptors (9, 14). Similar mechanisms are employed at the receptor locus to modulate m3 mAChR signaling (Figure 2). Rapid desensitization of the m3 mAChR, defined as a loss of agonist-stimulated G protein activation or phosphoinositide generation, has been observed in various cell types (15–17).

As is the case with many GPCRs, the m3 mAChR is subject to phosphorylation by GPCR kinases (GRKs) upon binding agonist (17–19). Phosphorylation by GRKs promotes receptor desensitization by partially uncoupling the receptor from G protein (reviewed in Refs. 20, 21). Interestingly, the m3 mAChR does not appear to be phosphorylated or regulated by protein kinase A or PKC (as are many other GPCRs), although a role for casein kinase 1 $\alpha$  in regulation of agonist-dependent m3 mAChR phosphorylation and desensitization has been demonstrated (22). Phosphorylation by GRKs promotes binding of arrestin molecules to the receptor, which more effectively uncouples the receptor from G protein by sterically inhibiting the receptor–G protein interaction. For numerous GPCRs, GRK-mediated arrestin binding also initiates receptor endocytosis/internalization or “sequestration” (from G protein), which occurs via the association of the receptor/arrestin complex with components of clathrin-coated pits (23). m3 mAChRs have been shown to undergo agonist-dependent sequestration in multiple cell types, although conflicting data exist regarding the role of arrestins in this process, with some studies suggesting that an arrestin-independent mechanism of m3 mAChR sequestration may exist (24, 25).

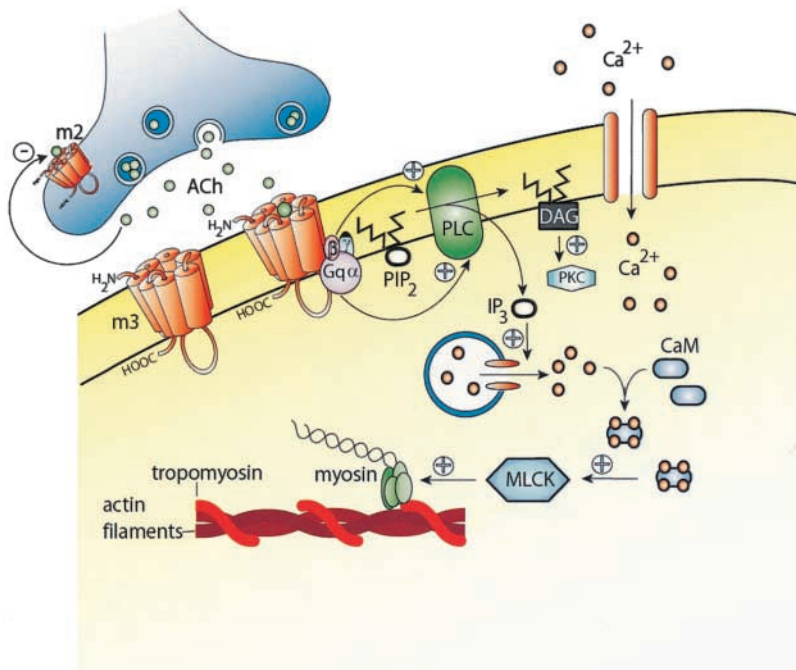
Sequestration is typically not a requirement for GPCR desensitization (sequestration can be blocked, yet receptors are desensitized by GRK-mediated phosphorylation and arrestin binding), but subjects the internalized receptor to potential fates that influence the magnitude of desensitization. Depending on the intensity and duration of agonist exposure, internalized receptors traffick primarily to two sorting pathways (26). One pathway involves receptor accumulation into lysosomes, which degrade receptor protein and thus reduce whole cell receptor density (*down-regulation*). Alternatively, receptors in early endosomes can be dephosphorylated and shunted to a pathway that promotes their return to the plasma membrane, where they can once again be activated (*recycling* contributing to *resensitization*). Therefore, the extent of receptor recycling and receptor degradation helps establish the level of cell surface receptors responsive to agonist. The m3 mAChR has been shown to undergo recycling (25) and downregulation (14, 27–29) in multiple cell types.

(Received in original form January 22, 2002)

Address correspondence to: Raymond B. Penn, Thomas Jefferson University, Kimmel Cancer Institute, Rm. 930 B.L.S.B., 233 S. 10th St., Philadelphia, PA 19107. E-mail: rpenn@lac.jci.tju.edu

**Abbreviations:** airway smooth muscle, ASM; intracellular calcium, [Ca<sup>2+</sup>]; calmodulin, CAM; 1,2 diacylglycerol, DAG; GPCR kinase, GRK; G protein-coupled receptors, GPCRs; inositol 1,4,5-trisphosphate, IP<sub>3</sub>; m3 muscarinic acetylcholine receptor, m3 mAChR; myosin light chain kinase, MLCK; protein kinase C, PKC; phospholipase C, PLC.

Am. J. Respir. Cell Mol. Biol. Vol. 26, pp. 269–272, 2002  
Internet address: www.atsjournals.org



**Figure 1.** m<sub>3</sub> mAChR-mediated signaling in ASM. Acetylcholine released from postganglionic parasympathetic nerves binds m<sub>3</sub> mAChRs on ASM and initiates a conformational change in the receptor that promotes its association with and activation of the heterotrimeric G protein G<sub>q</sub>. The activated  $\alpha$  subunit of G<sub>q</sub> in turn activates membrane-bound phospholipase C (PLC), which hydrolyzes phosphoinositol 4,5- bisphosphate (PIP<sub>2</sub>) into 1,2-diacylglycerol (DAG) and inositol 1,4,5- trisphosphate (IP<sub>3</sub>). IP<sub>3</sub> promotes Ca<sup>2+</sup> release from specialized intracellular compartments. Flux from voltage-dependent Ca<sup>2+</sup> channels (regulated by m<sub>3</sub> mAChR activation in an ill-defined manner [44]) also modulates intracellular Ca<sup>2+</sup> ([Ca<sup>2+</sup>]<sub>i</sub>) levels. DAG promotes the activation of protein kinase C (PKC) isoforms that phosphorylate numerous cellular enzymes that regulate diverse signaling events and cell functions, including altered [Ca<sup>2+</sup>]<sub>i</sub> and contraction. In ASM, PKC-mediated phosphorylation of actin-binding proteins such as calponin facilitates cross-bridge cycling. Increased [Ca<sup>2+</sup>]<sub>i</sub> induces the formation of Ca<sup>2+</sup>/calmodulin (CaM) complexes capable of activating myosin light chain kinase (MLCK). The subsequent phosphorylation of myosin light chain allows actin activation of myosin ATPase, cross-

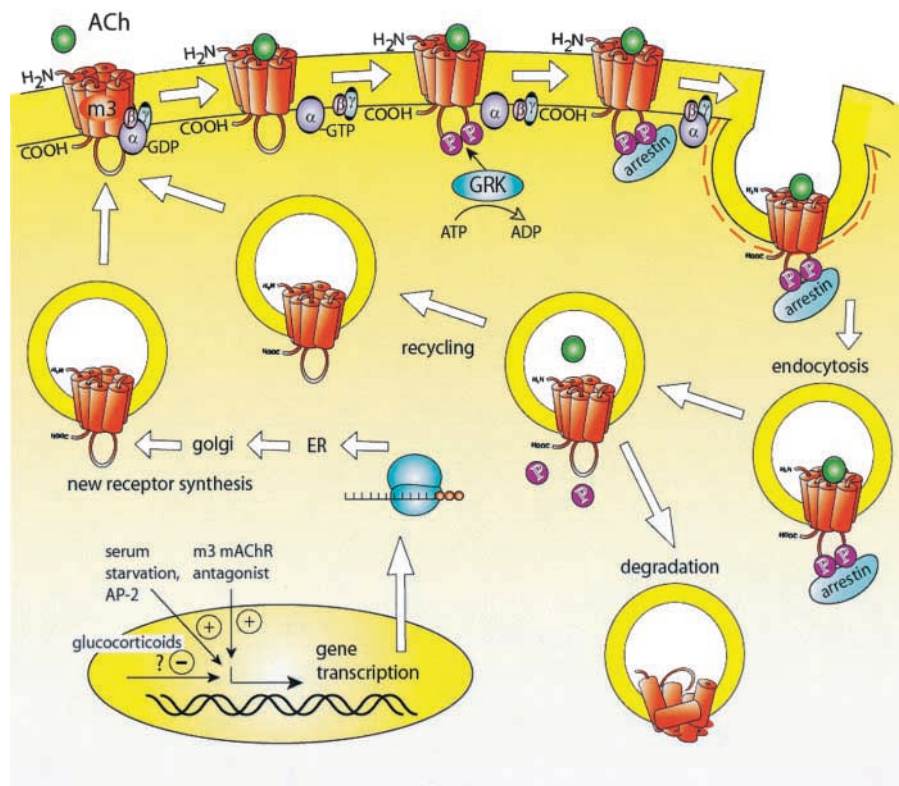
bridge cycling and the generation of force. Although other signaling pathways (e.g., involving activation of Rho, phospholipase D, and mitogen-activated protein kinases) have been shown to be mediated by the m<sub>3</sub> mAChR in various cell types, m<sub>3</sub> mAChR-G<sub>q</sub>-mediated PLC activation is the principal pathway regulating contraction in smooth muscle cells and secretion in most secretory cells. In ASM, a direct relationship between m<sub>3</sub> mAChR agonist-mediated phosphoinositide production and muscle contraction has been shown to exist (11). m<sub>3</sub> mAChR signaling can be regulated by either the degree of agonist presentation (altered cholinergic acetylcholine release, competition with antagonists) or by changes in the responsiveness of the transmembrane signaling proteins (*see text and Figure 2*).

Except in cases in which a reserve of “spare” receptors exists, alterations in cell surface GPCR density can significantly influence receptor signaling capacity. Cell surface GPCR density is, of course, determined in part by the rate of receptor protein synthesis, and thus the regulation of this synthesis by either transcriptional or post-transcriptional mechanisms represent a potentially important means of regulating receptor signaling. For the m<sub>3</sub> mAChR, such mechanisms regulating receptor synthesis are poorly understood.

In this month’s issue, Forsythe and coworkers report the structural organization of the m<sub>3</sub> mAChR gene and effectively jump-start investigation into the transcriptional regulation of the m<sub>3</sub> mAChR and its relevance to airway physiology (30). The > 285 kb gene contains 8 exons interrupted by 7 introns. Typical of most GPCRs cloned to date, the open reading frame is intronless. As in most other muscarinic receptor genes, the 5’ untranslated region contains no consensus TATA or CAAT boxes proximal to a cluster of transcriptional start sites. A luciferase reporter construct containing 1,240 bp of the 5’ untranslated sequence upstream of exon 1 exhibits considerable transcriptional activity when transfected in canine tracheal smooth muscle cells, and 5’ deletion analysis of this construct reveals positive regulatory elements in the region between -526 and -269 (relative to the most 5’ transcription start site). Interestingly, this region contains 3 AP-2 consensus binding motifs, and serum deprivation, previously shown to increase nuclear AP-2 levels in canine tracheal myocytes (31), results in a selective increase in tran-

scriptional activity. The role of AP-2 as an important positive regulator of m<sub>3</sub> mAChR gene transcription in ASM is further supported by experiments demonstrating that heterologous expression of AP-2 $\alpha$  also selectively increases transcriptional activity. A concurrent increase in m<sub>3</sub> mAChR protein with serum deprivation and AP-2 induction suggests m<sub>3</sub> mAChR expression is regulated predominantly by a transcriptional mechanism under these conditions. Conversely, in these cells, serum deprivation also increases SM22 and smooth muscle myosin heavy chain expression (32, 33), yet *inhibits* the transcriptional activity of their genes (31), establishing the importance of post-transcriptional mechanisms in regulating the expression of these proteins under these conditions.

The cloning of the m<sub>3</sub> mAChR gene is significant in that it provides a reductionist tool for determining whether any agents relevant to obstructive airway disease pathogenesis or its management have the capacity to influence m<sub>3</sub> mAChR expression (and therefore possibly resistance) in the airway. Indeed, some studies have suggested that changes in m<sub>3</sub> mAChR expression in the airway can influence disease severity or the efficacy of therapeutic agents, and that the impact of therapies on m<sub>3</sub> mAChR expression may be an important consideration in their design or application. Chronic infusion of the nonselective mAChR antagonist atropine into guinea pigs results in increased airway m<sub>3</sub> mAChR density with a concomitant increase in maximal methacholine-induced force generation in bronchial smooth muscle rings *ex vivo* (34), suggesting that up-regulation of m<sub>3</sub> mAChRs is the mechanism underlying



**Figure 2.** Regulation of the m3 mAChR. Regulation of m3 mAChR responsiveness upon receptor activation occurs via (i) receptor modification via phosphorylation by GPCR kinases (GRKs); (ii) changes in receptor localization that determine receptor-G protein compartmentalization as well as the kinetics of receptor recycling/degradation that establish levels of cell surface expression; and (iii) transcriptional and post-transcriptional mechanisms of receptor synthesis. Agonist binding induces a conformational change of the receptor promoting activation of the G-protein Gq and signaling but desensitization of transmembrane signaling is rapidly initiated by phosphorylation of the m3 mAChR 3rd intracellular loop by GRKs (17–19). Binding of arrestin to the phosphorylated receptor prevents further interaction between the receptor and Gq and promotes clathrin-mediated endocytosis. Internalized receptors traffic primarily to two sorting pathways. In the continued presence of agonist, receptors may enter into a lysosome-mediated degradation pathway. Alternatively, receptors in early endosomes can be dephosphorylated and recycle to the cell membrane. New receptor synthesis, reg-

ulated by various factors influencing the rate of gene transcription, mRNA stability, and translation, also determines the level of cell surface receptors responsive to agonist (see text for details).

increased bronchial hyperresponsiveness observed in patients with asthma treated chronically with ipratropium bromide (35, 36). Chronic mAChR antagonist treatment has also been shown to upregulate m3 mAChRs in rat hippocampus *in vivo* (37) and in cerebellar granule cells *in vitro*, the latter being associated with an increase in both m3 mAChR mRNA stability and gene transcription rate (38). Emala and colleagues (39) have demonstrated that chronic treatment of Basenji-greyhound dogs with glucocorticoids decreases m2 and m3 mAChR density in ASM. However, *in vitro* treatment of ASM cells with glucocorticoids has no effect on mAChR density, suggesting that the *in vivo* effect of glucocorticoids on ASM mAChR density requires a coregulatory molecule or is mediated by actions on a different cell type. Collectively, these studies suggest that m3 mAChR density can be dynamically regulated up or down by widely-administered therapies, and that such changes in expression may affect airway contractile state.

The role of altered m3 mAChR expression or responsiveness in obstructive airway disease pathogenesis is presently unestablished. Studies to date have tended to discount any role of m3 mAChR dysfunction *per se* in the development of hyperreactive airway disease (reviewed in Refs. 13, 40), and an initial screen suggests that any polymorphisms of the m3 mAChR coding sequence are rare (41). Instead, a convincing series of studies from the laboratories of Jacoby and Fryer suggests that exaggerated cholinergic discharge of acetylcholine, caused by a viral- or inflammation-driven inhibition of autoinhibitory m2 mAChRs expressed on postganglionic cholinergic nerves, contributes to in-

creased airway resistance in animal models (reviewed in Ref. 42) and most likely in certain populations of individuals with asthma (43). Yet it is important to note that obvious experimental limitations exist in accurately assessing features of airway receptor regulation *in vivo* in relevant human models (the jury has been out for over 25 years now as to whether  $\beta_2$  adrenergic receptor hyporesponsiveness contributes to asthma), such that current assessments of the role of m3 mAChR regulation in airway disease may be premature. The relatively poor selectivity of ligands and antibodies for mAChR subtypes renders such studies of the m3 mAChR particularly problematic. Ultimately, insight gained from *in vitro* analyses of the regulation of m3 mAChR gene expression and transmembrane signaling should inspire more integrative research that helps clarify how m3 mAChR activation and regulation influence obstructive airway diseases and their management.

*Acknowledgments:* The authors thank Christina Pao and You-Me Kim for assistance in generating figures.

**References**

1. Roffel, A. F., C. R. Elzinga, and J. Zaagsma. 1990. Muscarinic M3 receptors mediate contraction of human central and peripheral airway smooth muscle. *Pulm. Pharmacol.* 3:47–51.
2. Rogers, D. F. 2001. Motor control of airway goblet cells and glands. *Respir. Physiol.* 125:129–144.
3. Sato, E., S. Koyama, Y. Okubo, K. Kubo, and M. Sekiguchi. 1998. Acetylcholine stimulates alveolar macrophages to release inflammatory cell chemotactic activity. *Am. J. Physiol.* 274:L970–L979.
4. Bonner, T. I., N. J. Buckley, A. C. Young, and M. R. Brann. 1987. Identification of a family of muscarinic acetylcholine receptor genes. *Science* 237:527–532.
5. Peralta, E. G., A. Ashkenazi, J. W. Winslow, D. H. Smith, J. Ramachan-

- dran, and D. J. Capon. 1987. Distinct primary structures, ligand-binding properties and tissue-specific expression of four human muscarinic acetylcholine receptors. *EMBO J.* 6:3923-3929.
6. Budd, D. C., G. B. Willars, J. E. McDonald, and A. B. Tobin. 2001. Phosphorylation of the Gq/11-coupled m3-muscarinic receptor is involved in receptor activation of the ERK-1/2 mitogen-activated protein kinase pathway. *J. Biol. Chem.* 276:4581-4587.
  7. Bunemann, M., and M. M. Hosey. 2001. Novel signalling events mediated by muscarinic receptor subtypes. *Life Sci.* 68:2525-2533.
  8. Rosado, J. A., G. M. Salido, and L. J. Garcia. 2000. Activation of m3 muscarinic receptors induces rapid tyrosine phosphorylation of p125(FAK), p130(cas), and paxillin in rat pancreatic acini. *Arch. Biochem. Biophys.* 377:85-94.
  9. Schmidt, M., B. Fasselt, U. Rumenapp, C. Bienek, T. Wieland, C. J. van Koppen, and K. H. Jakobs. 1995. Rapid and persistent desensitization of m3 muscarinic acetylcholine receptor-stimulated phospholipase D: concomitant sensitization of phospholipase C. *J. Biol. Chem.* 270:19949-19956.
  10. Roffel, A. F., H. Meurs, C. R. Elzinga, and J. Zaagsma. 1990. Characterization of the muscarinic receptor subtype involved in phosphoinositide metabolism in bovine tracheal smooth muscle. *Br. J. Pharmacol.* 99:293-296.
  11. Meurs, H., A. F. Roffel, J. B. Postema, A. Timmermans, C. R. Elzinga, H. F. Kauffman, and J. Zaagsma. 1988. Evidence for a direct relationship between phosphoinositide metabolism and airway smooth muscle contraction induced by muscarinic agonists. *Eur. J. Pharmacol.* 156:271-274.
  12. Nahorski, S. R., A. B. Tobin, and G. B. Willars. 1997. Muscarinic M3 receptor coupling and regulation. *Life Sci.* 60:1039-1045.
  13. Zaagsma, J., A. F. Roffel, and H. Meurs. 1997. Muscarinic control of airway function. *Life Sci.* 60:1061-1068.
  14. van de Westerloo, E., J. Yang, C. Logsdon, and J. A. Williams. 1995. Down-regulation of the G-proteins Gq alpha and G11 alpha by transfected human M3 muscarinic acetylcholine receptors in Chinese hamster ovary cells is independent of receptor down-regulation. *Biochem. J.* 310:559-563.
  15. Bunday, R. A., and S. R. Nahorski. 2001. Homologous and heterologous uncoupling of muscarinic M(3) and alpha(1B) adrenoceptors to Galpha(q/11) in SH-SY5Y human neuroblastoma cells. *Br. J. Pharmacol.* 134:257-264.
  16. Tobin, A. B., D. G. Lambert, and S. R. Nahorski. 1992. Rapid desensitization of muscarinic m3 receptor-stimulated polyphosphoinositide responses. *Mol. Pharmacol.* 42:1042-1048.
  17. Willets, J. M., R. A. Challiss, E. Kelly, and S. R. Nahorski. 2001. G protein-coupled receptor kinases 3 and 6 use different pathways to desensitize the endogenous M3 muscarinic acetylcholine receptor in human SH-SY5Y cells. *Mol. Pharmacol.* 60:321-330.
  18. Hosey, M. M., J. L. Benovic, S. K. DebBurman, and R. M. Richardson. 1995. Multiple mechanisms involving protein phosphorylation are linked to desensitization of muscarinic receptors. *Life Sci.* 56:951-955.
  19. Wu, G., G. S. Bogatkevich, Y. V. Mukhin, J. L. Benovic, J. D. Hildebrandt, and S. M. Lanier. 2000. Identification of Gbetagamma binding sites in the third intracellular loop of the M(3)-muscarinic receptor and their role in receptor regulation. *J. Biol. Chem.* 275:9026-9034.
  20. Penn, R. B., and J. L. Benovic. 1998. Regulation of G protein-coupled receptors. In *Handbook of Physiology*. P. M. Conn, editor. Oxford University Press. 125-164.
  21. Penn, R. B., A. P. Pronin, and J. L. Benovic. 2000. Regulation of G protein-coupled receptor kinases. *Trends Cardiovasc. Med.* 10:81-89.
  22. Budd, D. C., J. E. McDonald, and A. B. Tobin. 2000. Phosphorylation and regulation of a Gq/11-coupled receptor by casein kinase 1alpha. *J. Biol. Chem.* 275:19667-19675.
  23. Goodman, O. B., Jr., J. G. Krupnick, F. Santini, V. V. Gurevich, R. B. Penn, A. W. Gagnon, J. H. Keen, and J. L. Benovic. 1996. Beta-arrestin acts as a clathrin adaptor in endocytosis of the beta2- adrenergic receptor. *Nature* 383:447-450.
  24. Lee, K. B., R. Pals-Rylaarsdam, J. L. Benovic, and M. M. Hosey. 1998. Arrestin-independent internalization of the m1, m3, and m4 subtypes of muscarinic cholinergic receptors. *J. Biol. Chem.* 273:12967-12972.
  25. Edwardson, J. M., and P. G. Szekeres. 1999. Endocytosis and recycling of muscarinic receptors. *Life Sci.* 64:487-494.
  26. Tsao, P., T. Cao, and M. von Zastrow. 2001. Role of endocytosis in mediating downregulation of G-protein-coupled receptors. *Trends Pharmacol. Sci.* 22:91-96.
  27. Yang, J., C. D. Logsdon, T. E. Johansen, and J. A. Williams. 1993. Human m3 muscarinic acetylcholine receptor carboxy-terminal threonine residues are required for agonist-induced receptor down-regulation. *Mol. Pharmacol.* 44:1158-1164.
  28. Shockley, M. S., L. M. Tolbert, A. B. Tobin, S. R. Nahorski, W. Sadee, and J. Lameh. 1999. Differential regulation of muscarinic M1 and M3 receptors by a putative phosphorylation domain. *Eur. J. Pharmacol.* 377:137-146.
  29. Detjen, K., J. Yang, and C. D. Logsdon. 1995. Muscarinic acetylcholine receptor down-regulation limits the extent of inhibition of cell cycle progression in Chinese hamster ovary cells. *Proc. Natl. Acad. Sci. USA* 92:10929-10933.
  30. Forsythe, S. M., P. C. Kogut, J. F. McConville, Y. Fu, J. A. McCauley, A. J. Halayko, H. W. Liu, A. Kao, D. J. Fernandes, S. Bellam, E. Fuchs, S. Sinha, G. I. Bell, B. Camovetti-Mercado, and J. Solway. 2002. Structure and transcription of the human m3 muscarinic receptor gene. *Am. J. Respir. Cell Mol. Biol.* 26:298-305.
  31. Camoretti-Mercado, B., H. W. Liu, A. J. Halayko, S. M. Forsythe, J. W. Kyle, B. Li, Y. Fu, J. McConville, P. Kogut, J. E. Vieira, N. M. Patel, M. B. Hershenson, E. Fuchs, S. Sinha, J. M. Miano, M. S. Parmacek, J. K. Burkhardt, and J. Solway. 2000. Physiological control of smooth muscle-specific gene expression through regulated nuclear translocation of serum response factor. *J. Biol. Chem.* 275:30387-30393.
  32. Ma, X., Y. Wang, and N. L. Stephens. 1998. Serum deprivation induces a unique hypercontractile phenotype of cultured smooth muscle cells. *Am. J. Physiol.* 274:C1206-C1214.
  33. Halayko, A. J., B. Camoretti-Mercado, S. M. Forsythe, J. E. Vieira, R. W. Mitchell, M. E. Wylam, M. B. Hershenson, and J. Solway. 1999. Divergent differentiation paths in airway smooth muscle culture: induction of functionally contractile myocytes. *Am. J. Physiol.* 276:L197-L206.
  34. Witt-Enderby, P. A., H. I. Yamamura, M. Halonen, J. Lai, J. D. Palmer, and J. Bloom. 1995. Regulation of airway muscarinic cholinergic receptor subtypes by chronic anticholinergic treatment. *Mol. Pharmacol.* 47:485-490.
  35. van Schayck, C. P., E. Dompeling, C. L. van Herwaarden, H. Folgering, A. L. Verbeek, H. J. van der Hoogen, and C. van Weel. 1991. Bronchodilator treatment in moderate asthma or chronic bronchitis: continuous or on demand? A randomised controlled study. *BMJ* 303:1426-1431.
  36. Newcomb, R., D. P. Tashkin, K. K. Hui, M. E. Conolly, E. Lee, and B. Dauphinee. 1985. Rebound hyperresponsiveness to muscarinic stimulation after chronic therapy with an inhaled muscarinic antagonist. *Am. Rev. Respir. Dis.* 132:12-15.
  37. Wall, S. J., R. P. Yasuda, M. Li, W. Ciesla, and B. B. Wolfe. 1992. Differential regulation of subtypes m1-m5 of muscarinic receptors in forebrain by chronic atropine administration. *J. Pharmacol. Exp. Ther.* 262:584-588.
  38. Fukumachi, F., P. A. Saunders, C. Hough, and D. M. Chuang. 1993. Agonist-induced down-regulation and antagonist-induced up-regulation of m2- and m3-muscarinic acetylcholine receptor mRNA and protein in cultured cerebellar granule cells. *Mol. Pharmacol.* 44:940-949.
  39. Emala, C. W., J. Clancy, and C. A. Hirshman. 1997. Glucocorticoid treatment decreases muscarinic receptor expression in canine airway smooth muscle. *Am. J. Physiol.* 272:L745-L751.
  40. Fryer, A. D., and D. B. Jacoby. 1998. Muscarinic receptors and control of airway smooth muscle. *Am. J. Respir. Crit. Care Med.* 158:S154-S160.
  41. Fenech, A. G., M. J. Ebejer, A. E. Felice, R. Ellul-Micallef, and I. P. Hall. 2001. Mutation screening of the muscarinic M(2) and M(3) receptor genes in normal and asthmatic subjects. *Br. J. Pharmacol.* 133:43-48.
  42. Costello, R. W., D. B. Jacoby, and A. D. Fryer. 1998. Pulmonary neuronal M2 muscarinic receptor function in asthma and animal models of hyperreactivity. *Thorax* 53:613-616.
  43. Minette, P. A., J. W. Lammers, C. M. Dixon, M. T. McCusker, and P. J. Barnes. 1989. A muscarinic agonist inhibits reflex bronchoconstriction in normal but not in asthmatic subjects. *J. Appl. Physiol.* 67:2461-2465.
  44. Webb, B. L., S. J. Hirst, and M. A. Giembycz. 2000. Protein kinase C isoenzymes: a review of their structure, regulation and role in regulating airways smooth muscle tone and mitogenesis. *Br. J. Pharmacol.* 130:1433-1452.