Current Neurobiology 2011; 2 (1): 23-29

IFNγ Increases M2 Muscarinic Receptor Expression in Cultured Sympathetic Neurons

Ana Cristina G. Grodzki[1], Atefeh Ghogha[1], Linley Mangini[1], Allison D. Fryer[2] and Pamela J. Lein[1]

[1]Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis, CA, USA
[2]Department of Pulmonary and Critical Care, Oregon Health & Sciences University, Portland, OR, USA

Abstract

M2 muscarinic receptors are expressed on both parasympathetic and sympathetic nerve endings where they function as autoinhibitory receptors to limit release of acetylcholine and norepinephrine, respectively. M2 muscarinic receptor expression on parasympathetic nerves is decreased by viral infection and by gamma-interferon (IFNγ) and increased by dexamethasone; and these effects are of clinical relevance in the etiology and treatment of asthma. Whether IFNγ and dexamethasone similarly modulate M2 receptor expression on sympathetic nerves is not known. To address this question, we examined the effects of IFNγ and dexamethasone on M2 receptor expression at the mRNA and protein level in primary cultures of sympathetic neurons dissociated from the rat superior cervical ganglia (SCG). Semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) indicated that neither IFNγ nor dexamethasone altered M2 receptor transcript levels. However, western blot analyses demonstrated that IFNγ, but not dexamethasone, increases M2 receptor protein expression in sympathetic neurons. Increased expression did not significantly alter subcellular localization of M2 receptors in sympathetic neurons as determined using immunocytochemistry. These findings indicate that M2 receptors are differentially regulated in different types of autonomic neurons, and they suggest a novel mechanism by which IFNγ may contribute to airway hyperreactivity in viral-induced asthma.

Keywords: IFNγ, dexamethasone, M2 muscarinic receptor, sympathetic nerves.

Accepted February 22 2011

Introduction

Airway tone is controlled by the autonomic nervous system, and in humans, airways are predominantly innervated by the parasympathetic nervous system. Postganglionic parasympathetic nerves release acetylcholine, which activates postsynaptic receptors to cause smooth muscle contraction and mucus secretion [1]. These actions are opposed by neurons of the sympathetic nervous system, which synapse on parasympathetic ganglia and nerve terminals [2, 3]. The release of norepinephrine by sympathetic neurons at either of these synaptic sites inhibits acetylcholine release from parasympathetic nerves [4], thereby reducing parasympathetic-mediated airway bronchoconstriction (Fig. 1). Thus, even though sympathetic neurons do not directly innervate airway smooth muscle in humans, the sympathetic nervous system can play a significant functional role in the human respiratory system by inhibiting parasympathetic neurotransmission. Understanding how sympathetic neurotransmission is modulated may provide important insights into the pathogenesis or treatment of asthma and other respiratory diseases characterized by clinically significant bronchoconstriction. Decreased sympathetic nerve activity would be expected to exacerbate airway hyperreactivity and secretions via increased parasympathetic activity. Conversely, enhanced sympathetic activity could potentially relieve asthmatic symptoms by decreasing parasympathetic neurotransmission.

Neurotransmitter release from parasympathetic nerves is controlled by autoinhibitory M2 muscarinic receptors on parasympathetic neurons. Activation of these neuronal M2 receptors restricts acetylcholine release limiting bronchoconstriction [6, 7]. In parasympathetic nerves, M2 receptor expression is modulated by pro- and anti-inflammatory agents associated with asthma. Specifically, the pro-inflammatory cytokine gamma-interferon (IFNγ) downregulates M2 receptor expression, resulting in increased acetylcholine release that may contribute to the airway hyperreactivity associated with virally-induced asthma [8]. In contrast, dexamethasone, an anti-

inflammatory glucocorticoid increases M2 receptor expression in parasympathetic neurons and this may contribute to the therapeutic efficacy of steroids in treatment of asthma [9]. Sympathetic neurons also express M2 muscarinic receptors that decrease norepinephrine release [5]. However, it is not known whether IFNγ and dexamethasone similarly alter M2 receptor expression in sympathetic neurons. To address this question, we examined the effects of IFNγ and dexamethasone on M2 receptor expression at the mRNA and protein levels in primary cultures of sympathetic neurons dissociated from rat superior cervical ganglia (SCG). Our data demonstrate that IFNγ but not dexamethasone modulates M2 receptor expression in sympathetic neurons. In contrast to previous observations of parasympathetic nerves, IFNγ increased M2 receptor protein levels in sympathetic nerves. These findings indicate that M2 muscarinic receptors are differentially regulated in different autonomic neurons, and suggest a novel mechanism by which IFNγ may contribute to airway hyperreactivity in viral-induced asthma.

Materials and Methods

Materials

Stock solution of purified recombinant rat IFNγ (PreproTech; Rocky Hill, NJ) was prepared at 2μg/ml in phosphate buffered saline (PBS). Stock solution of dexamethasone (Sigma Aldrich; St. Louis, MO; with specific activity of 6.3%) was prepared at 2mM in PBS. Stocks were stored at -80°C, thawed immediately prior to use, and added directly to tissue culture medium. Purified human recombinant bone morphogenetic protein-7 (BMP-7) was generously provided by Curis (Cambridge, MA).

Animals

All procedures and protocols involving animals were approved by the Institutional Animal Care and Use Committees of the University of California, Davis. Timed-pregnant Sprague Dawley rats were purchased from Charles River Laboratories (Hollister, CA) and housed individually in standard plastic cages with Alpha-Dri bedding (Shepherd Specialty Papers, Watertown, TN) in a temperature (22±2°C) controlled room on a 12h reverse light-dark cycle. Food and water were provided *ad libitum*. All animals were euthanized prior to harvesting of tissue for culture.

Cell culture

Sympathetic neurons were dissociated from the superior cervical ganglia (SCG) of embryonic day 21 rats according to previously described methods [10]. Cells were plated onto glass coverslips or 6 well plates pre-coated with 100μg/ml poly-D-lysine (Sigma). Cultures were maintained in serum-free medium supplemented with β-NGF (100ng/ml, Harlan Bioproducts, Indianapolis, IN), bovine serum albumin (500μg/ml, Invitrogen, Carlsbad, CA) and insulin-transferrin-selenium (10μg/ml, 5.5μg/ml and 38.7nM, respectively, Invitrogen). To eliminate endogenous non-neuronal cells, cytosine-β-D-arabinofuranoside (ARA-C, Sigma) was added to the culture medium at 1μM for 48h beginning on day 2. In a subset of cultures, ARA-C was not added to allow endogenous non-neuronal cells to proliferate. Previous studies have demonstrated that under these culture conditions, the non-neuronal cells are primarily ganglionic glia [11]. To stimulate dendritic growth, cultures were treated with BMP-7 (50ng/ml) beginning on day 5 *in vitro* [12]. After 2 weeks in culture, cells were treated with IFNγ (30ng/ml) or dexamethasone (1μM) for 24h.

RNA Isolation and Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Total RNA was extracted from cultured sympathetic neurons using the Qiagen RNAeasy Mini Kit (Qiagen Inc., Valencia, CA). The quality and concentration of extracted RNA were evaluated using the Nanodrop 1000 (Thermo Scientific, Rockford, IL, USA). All samples were of high purity (ratio 260/280 > 2). RNA samples (0.5μg) were reverse transcribed using SuperScript® III Reverse Transcriptase (Invitrogen) and random primers at an annealing temperature of 65°C. Resultant cDNA was amplified by PCR for 35 cycles using an annealing temperature of 55°C for 1min and denaturing temperature of 94°C for 15sec. Preliminary tests examining the relationship between cycle number and product formation confirmed that under these conditions 35 cycles was in the linear portion of the response curve with the response reaching a plateau at 40 cycles. Primers obtained from Integrated DNA Technologies, Inc. (San Diego, CA) were used to amplify M2 muscarinic receptor transcripts and 18S rRNA and were designed using PrimerQuest program (Integrated DNA Technologies). The specific sequences of the M2 receptor primers were 5'-ATC CCG GGC AAG CAA GAG TAG AAT-3' and 5'-TCA CCG TGT AGC GCC TAT GTT CTT-3'; the specific sequences for the 18S rRNA primers were 5'-CCA GAG CGA AAG CAT TTG CCA AGA-3' AND 5'- AAT CAA CGC AAG CTT ATG ACC CGC-3'. After synthesis, PCR products were subjected to 1.2% agarose gel electrophoresis to confirm the expected size of 775 base pairs for M2 receptor RT-PCR products and 689 base pairs for 18S rRNA RT-PCR products. The density of each band was quantified using the Kodak gel image system (Kodak, Rochester, NY) and the values obtained for the M2 receptor were normalized to the values obtained for 18S rRNA for the same sample.

Immunocytochemistry

After 2 weeks *in vitro*, cultures were fixed in methanol at -20°C for 5min, rinsed 3 times with PBS and blocked in

PBS with 5% BSA for 20min. Cultures were then incubated with rabbit anti-muscarinic M2 receptor (1:500; Research & Diagnostic Antibodies, Las Vegas, NV) for 1h at room temperature, rinsed 3 times with PBS and incubated with goat anti-rabbit IgG conjugated to Alexa488 (1:500; Invitrogen) for 30min at room temperature. After rinsing 3 times with PBS, the cells were rinsed once with distilled water and mounted onto glass slides using Vectashield (Vector Laboratories, Burlingame, CA). Cells were analyzed using an Olympus IX81 confocal microscope equipped with a cooled CCD camera (Olympus America Inc., Center Valley, PA).

Western blot analyses

Western blot analyses were performed on cell lysates of cultured sympathetic neurons to assess the effects of dexamethasone and IFNγ on M2 muscarinic receptor expression at the protein level. Cell lysates were obtained by rinsing 2 week-old cultures with ice-cold PBS prior to trituration in ice-cold lysis PBS supplemented with 1% Igepal (Sigma), 0.5% sodium deoxycholate (FisherScientific, Fair Lawn, NJ), 0.1% SDS (Sodium Dodecyl Sulfate; FisherScientific), 100µg/ml PMSF (Phenylmethylsulfonyl Fluoride; Sigma) and 300µg/ml aprotinin (Sigma). Cell lysates were centrifuged in an Eppendorf 5417R microfuge (Eppendorf, Hauppauge, NY) at maximum speed for 5min and the resultant supernatant collected. Protein concentration was determined using the micro BCA™ Protein Assay Kit (Thermo Scientific, Rockford, IL, USA). Samples (25µg total protein) were resolved by 12% SDS polyacrylamide gel electrophoresis and electroblotted onto Immobilon-FL membranes (Millipore, Bedford, MA). Blots were blocked at room temperature for 1h in Odyssey blocking buffer (LI-COR, Biosciences, Lincoln, NE) diluted 1:1 with PBS, then incubated overnight at 4°C in Odyssey blocking buffer diluted 1:1 with PBS containing 0.1% tween 20 and anti-M2 antibody (1:2000; Sigma) and anti-α tubulin antibody (1:10,000; Sigma). Blots were washed 4 times with PBS containing 0.1% tween 20 and incubated at room temperature for 1h in Odyssey blocking buffer diluted 1:1 with PBS containing 0.1% tween 20 and goat anti-rabbit infrared (IR)700 diluted 1:1,000 and goat anti-mouse IR800 diluted 1:20,000. Subsequently, blots were washed 4 times as described above, visualized and quantified using the Odyssey Infrared Imaging system (LI-COR Biosciences).

Statistical analysis

All data are presented as the mean ± SEM. Data were analyzed by *ANOVA* for treatment effects using GraphPad Prism 4 software (San Diego, CA). If significant effects were identified ($p < 0.05$), *post-hoc* analyses were performed using the Tukey's test.

Results

IFNγ and dexamethasone do not alter M2 mRNA levels in cultured sympathetic neurons

We used semi-quantitative RT-PCR to quantify M2 receptor transcripts in cultured sympathetic neurons treated with IFNγ at 30ng/ml and dexamethasone at 1µM for 24h. In sympathetic neurons cultured in the absence of ganglionic glial cells, neither IFNγ nor dexamethasone significantly altered M2 mRNA levels relative to control cultures treated with vehicle (Fig. 2A, 2B). Because IFNγ and dexamethasone alter M2 receptor mRNA expression

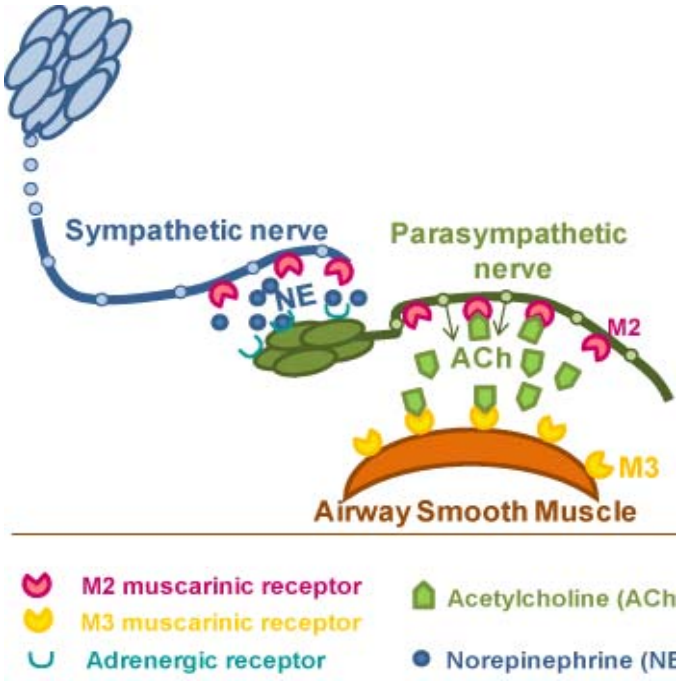

Figure 1. Regulation of airway caliber by parasympathetic and sympathetic nervous systems. *Acetylcholine released from parasympathetic nerves activates postsynaptic M3 muscarinic receptors resulting in contraction of airway smooth muscle and bronchoconstriction. ACh also binds to presynaptic M2 muscarinic receptors on parasympathetic nerves to limit further ACh release from parasympathetic nerves. Release of ACh from parasympathetic nerves is also inhibited by norepinephrine (NE) released from postganglionic sympathetic nerves, which binds to adrenergic receptors on parasympathetic neurons. Sympathetic nerves also express M2 receptors that inhibit neurotransmitter release. Thus, there is the potential that acetylcholine released from parasympathetic nerves inhibit sympathetic transmission, and by inhibiting norepinephrine release, indirectly increase acetylcholine release. Increased ACh release increases parasympathetic-mediated bronchoconstriction.*

in parasympathetic neurons [8, 9] that are grown with glial and other non-neuronal cells, we also tested sympa-

thetic neurons cultured in the presence of endogenous ganglionic glia cells. Neither IFNγ nor dexamethasone significantly changed M2 receptor mRNA levels in these neuron-glia co-cultures relative to vehicle controls (Fig. 2C, 2D).

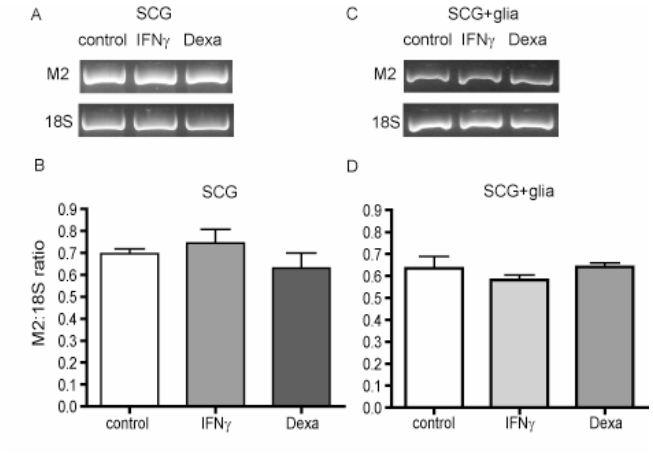

Figure 2. Dexamethasone and IFNγ do not alter M2 receptor mRNA levels in cultured sympathetic neurons. *Sympathetic neurons were cultured in the absence (A, B) or presence (C, D) of ganglionic glia. Cultures were exposed to IFNγ (30ng/ml), dexamethasone (Dexa, 1μM) or vehicle (control) for 24h prior to extraction of total RNA. Shown are representative gels of RT-PCR products generated using primers specific for M2 muscarinic receptors or 18s rRNA (A, C) and densitometric analyses of M2 receptor mRNA expression normalized to 18s rRNA expression (B, D). Data presented are the mean +/- SEM (n = 3 independent experiments). No statistically significant treatment effects were detected by one-way ANOVA.*

IFNγ and dexamethasone do not significantly alter subcellular localization of M2 receptors in cultured sympathetic neurons

In sympathetic neurons grown in the absence of ganglionic non-neuronal cells, M2 receptor immunoreactivity was observed throughout the extranuclear compartment of the soma and throughout the neuritic complex (Fig. 3A). Quantification of fluorescence intensity demonstrated no effect of IFNγ or dexamethasone on M2 receptor expression (data not shown) or localization (Fig. 3B, 3C) in these cultured neurons. In mixed cultures of sympathetic neurons and ganglionic glial cells, M2 immunoreactivity was associated with not only neurons but also glial cells (Fig. 3D). Co-culturing with ganglionic glial cells did seem to cause subtle changes in M2 receptor immunoreactivity in neurons evident as a more punctuate pattern of immunofluorescence in neurites (Fig. 3D versus Fig. 3A).

However, as observed in cultures of purified sympathetic neurons, exposure to IFNγ or dexamethasone did not significantly change either the fluorescence intensity (data not shown) or the subcellular distribution of M2 receptor immunoreactivity in neuron-glia co-cultures (Fig. 3E, 3F).

IFNγ increases M2 protein expression in cultured sympathetic neurons

We used western blotting to quantify M2 receptor expression in cell cultures exposed to IFNγ and dexamethasone for 24h. Cell lysates from purified sympathetic neuronal cell cultures and from neuron-glia co-cultures were separated using SDS PAGE and then immunoblotted for M2 receptor and α-tubulin. In sympathetic neurons cultured in the absence of glia, IFNγ significantly increased M2 receptor expression; however, dexamethasone had no effect (Fig. 4). However, in neuron-glia co-cultures neither treatment caused a significant change in levels of M2 receptor expression (data not shown).

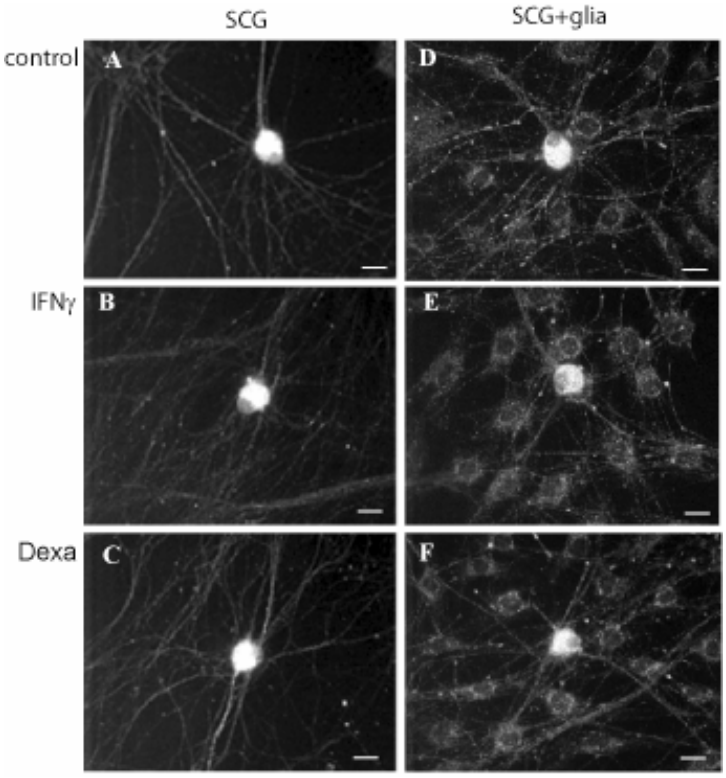

Figure 3. The subcellular localization of M2 muscarinic receptors in cultured sympathetic neurons is not altered by exposure to IFNγ or dexamethasone. *Sympathetic neurons were cultured in the absence (A, B, C) or presence (D, E, F) of ganglionic glia. After 2 wk in vitro, cultures were exposed to IFNγ (30ng/ml) (B, E) or dexamethasone (Dexa, 1μM) (C, F) for 24h, then fixed and immunostained to localize M2 muscarinic receptors. Quantification of fluorescence did not indicate treatment-related differences in intensity of staining (data not shown). Photomicrographs are representative of data obtained from 3 independent experiments. Scale bar = 20μm.*

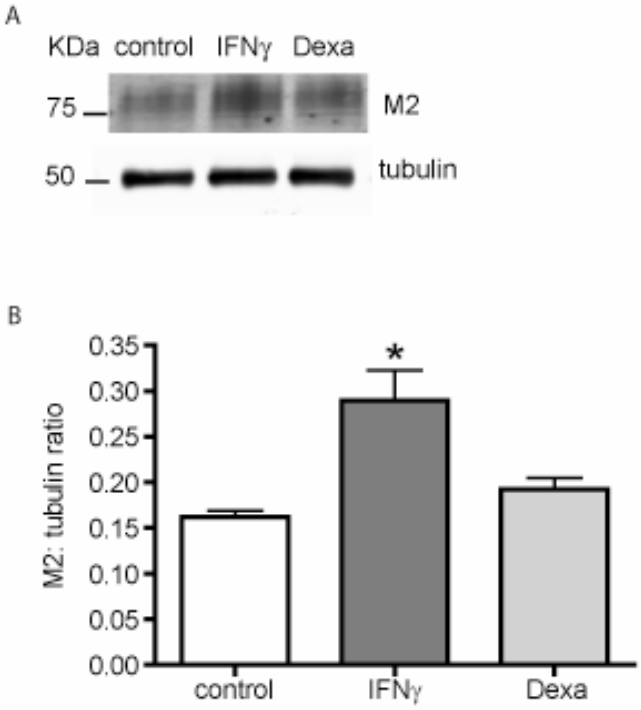

Figure 4. IFNγ but not dexamethasone increases M2 receptor protein levels in cultured sympathetic neurons. *Sympathetic neurons were dissociated from perinatal rat superior cervical ganglia (SCG) and non-neuronal cells were eliminated using an anti-mitotic agent. After 2 wk in vitro, cultures were exposed to IFNγ (30ng/ml), dexamethasone (Dexa, 1μM) or vehicle (control) for 24h prior to collection of cell lysates for western blot analyses. (A) Representative blot of cell lysates separated by gel electrophoresis then immunoblotted for M2 or α-tubulin. (B) Densitometric analyses of M2 receptor levels were normalized to α-tubulin levels. Data are presented as the mean ± SEM (n = 3 per experimental condition). *Significantly different from control at p<0.05 by 1 way ANOVA followed by post hoc Tukey's test.*

Discussion

Both branches of the autonomic nervous system innervate airways: parasympathetic nerves, which decrease airway diameter directly via release of acetylcholine onto postsynaptic M3 muscarinic receptors, and sympathetic nerves, which inhibit release of acetylcholine from parasympathetic nerves. Clinical and experimental evidence demonstrate that loss of autoinhibitory M2 receptor function on parasympathetic nerves contributes to airway hyperreactivity associated with asthma and other respiratory diseases, and that restoration of M2 receptor function prevents airway hyperreactivity [13], [14-16]. IFNγ, a pro-inflammatory cytokine implicated in the pathogenesis of asthma [17, 18], decreases levels of M2 receptor mRNA in parasympathetic neurons, and this effect is thought to mediate virally-induced asthma [8]. Conversely, dexamethasone, an anti-inflammatory glucocorticoid, has been demonstrated to decrease airway responsiveness in animal models of asthma by increasing M2 receptor expression and function in parasympathetic nerves [9]. Here we tested whether IFNγ and dexamethasone similarly regulate M2 receptor expression in sympathetic neurons.

Our findings demonstrate that in primary neuronal cell cultures derived from rat SCG, dexamethasone, at the same concentration and for the same exposure time used in prior studies of parasympathetic nerve cell cultures [8, 9], had no effect on M2 receptor expression as determined by RT-PCR, immunocytochemistry and western blotting. IFNγ had no effect on M2 receptor transcript levels in cultured sympathetic neurons, but did significantly increase M2 receptor expression at the protein level in purified neuronal cell cultures; however, this did not cause significant changes in the subcellular localization of M2 receptors as determined by immunocytochemistry. It may be important that IFNγ did not upregulate M2 receptor expression in neuron-glia co-cultures. Glial cells can alter the response of neurons to various environmental stressors [19], so it is possible that co-culture with ganglionic glial cells altered the neuronal response to IFNγ. However, ganglionic glial cells also express M2 receptors [20, 21], as confirmed in our immunocytochemical analyses of neuron-glia co-cultures, and after 14 days in culture, glial cells significantly outnumber neurons by several orders of magnitude in SCG neuron-glia co-cultures. Thus, it is likely the different results obtained in western blot analyses of purified neuronal cell cultures *versus* neuron-glia co-cultures is due in large part to the fact that the majority of M2 receptor protein in glia-neuron co-cultures is glial and the effects of IFNγ on neuronal M2 receptor expression were masked by the overwhelming glial M2 receptor signal. Regardless, an outstanding question raised by the observation that IFNγ is increasing M2 receptor protein in sympathetic neurons concerns the mechanism(s) underlying this effect. Our data support post-transcriptional mechanisms, which is consistent with previous reports of IFNγ regulation of nitric oxide synthetase expression [22].

The reason(s) for the differential effects of dexamethasone and IFNγ on sympathetic versus parasympathetic neurons are not known. One potential factor we considered was that non-neuronal cells present in the parasympathetic nerve cell cultures [8, 9], may have influenced the effect of dexamethasone and IFNγ on neuronal M2 receptor expression. To address this possibility, we examined the effects of IFNγ and dexamethasone on sympathetic neurons co-cultured with ganglionic non-neuronal cells, which are primarily glia [11]. However, inclusion of ganglionic glial cells did not modify the M2 receptor response of sympathetic neurons to more closely replicate that of parasympathetic nerve cell cultures. It is possible that other non-neuronal cells, present in parasympathetic nerve cultures and absent from sympathetic

neuron-glia co-cultures, mediate the effect of IFNγ and dexamethasone on M2 receptor expression in parasympathetic nerves. Alternatively, since parasympathetic nerves were derived from guinea pig trachea while sympathetic nerves were derived from rat SCG, the differential response between the two autonomic nerve cell types may reflect species-specific differences. However, it seems more likely that our findings demonstrate fundamental differences in the pharmacology of parasympathetic and sympathetic neurons.

The physiological significance of IFNγ-induced M2 receptor expression in postganglionic sympathetic neurons has yet to be determined. However, we predict that upregulation of M2 receptor expression would decrease norepinephrine release from sympathetic neurons, relieving sympathetic suppression of parasympathetic neurotransmission. This, in turn, would increase release of acetylcholine from parasympathetic nerves, exacerbating airway hyperreactivity. Because IFNγ is a key cytokine stimulated by viral infection [8], these data suggests a novel mechanism that may contribute to virally-induced airway hyperreactivity.

Acknowledgements

This work was supported by the National Institutes of Health (grant R01 ES017592 to PJL and ADF, and a postdoctoral fellowship awarded to A. Grodzki through grant T32 HL007013). The funding agencies had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

1. Belvisi MG: Overview of the innervation of the lung. Curr Opin Pharmacol 2002; 2:211-5.
2. van der Velden VH, Hulsmann AR: Autonomic innervation of human airways: structure, function, and pathophysiology in asthma. Neuroimmunomodulation 1999; 6:145-59.
3. Reekie FM, Burnstock G: Effects of noradrenaline on rat paratracheal neurones and localization of an endogenous source of noradrenaline. Br J Pharmacol 1992; 107:471-5.
4. Baker DG, Don HF, Brown JK: Direct measurement of acetylcholine release in guinea pig trachea. Am J Physiol 1992; 263:L142-7.
5. Pendry YD, Maclagan J: Evidence for inhibition of sympathetic neurotransmission by endogenously released acetylcholine in the guinea-pig trachea. Br J Pharmacol 1991; 104:817-22.
6. Fryer AD, Maclagan J: Muscarinic inhibitory receptors in pulmonary parasympathetic nerves in the guinea-pig. Br J Pharmacol 1984; 83:973-8.
7. Minette PA, Barnes PJ: Prejunctional inhibitory muscarinic receptors on cholinergic nerves in human and guinea pig airways. J Appl Physiol 1988; 64:2532-7.
8. Jacoby DB, Xiao HQ, Lee NH, Chan-Li Y, Fryer AD: Virus- and interferon-induced loss of inhibitory M2 muscarinic receptor function and gene expression in cultured airway parasympathetic neurons. J Clin Invest 1998; 102:242-8.
9. Jacoby DB, Yost BL, Kumaravel B, Chan-Li Y, Xiao HQ, Kawashima K, Fryer AD: Glucocorticoid treatment increases inhibitory m(2) muscarinic receptor expression and function in the airways. Am J Respir Cell Mol Biol 2001; 24:485-91.
10. Kim IJ, Beck HN, Lein PJ, Higgins D: Interferon gamma induces retrograde dendritic retraction and inhibits synapse formation. J Neurosci 2002; 22:4530-9.
11. Tropea M, Johnson MI, Higgins D: Glial cells promote dendritic development in rat sympathetic neurons in vitro. Glia 1988; 1:380-92.
12. Lein P, Johnson M, Guo X, Rueger D, Higgins D: Osteogenic protein-1 induces dendritic growth in rat sympathetic neurons. Neuron 1995; 15:597-605.
13. Minette PA, Lammers JW, Dixon CM, McCusker MT, Barnes PJ: A muscarinic agonist inhibits reflex bronchoconstriction in normal but not in asthmatic subjects. J Appl Physiol 1989; 67:2461-5.
14. Ayala LE, Ahmed T: Is there loss of protective muscarinic receptor mechanism in asthma? Chest 1989; 96:1285-91.
15. Fryer AD, Wills-Karp M: Dysfunction of M2-muscarinic receptors in pulmonary parasympathetic nerves after antigen challenge. J Appl Physiol 1991; 71:2255-61.
16. Verbout NG, Lorton JK, Jacoby DB, Fryer AD: Atropine pretreatment enhances airway hyperreactivity in antigen-challenged guinea pigs through an eosinophil-dependent mechanism. Am J Physiol Lung Cell Mol Physiol 2007; 292:L1126-35.
17. Yang M, Kumar RK, Foster PS: Pathogenesis of steroid-resistant airway hyperresponsiveness: interaction between IFN-gamma and TLR4/MyD88 pathways. J Immunol 2009; 182:5107-15.
18. Kumar RK, Webb DC, Herbert C, Foster PS: Interferon-gamma as a possible target in chronic asthma. Inflamm Allergy Drug Targets 2006; 5:253-6.
19. . Kraft AD, Johnson DA, Johnson JA: Nuclear factor E2-related factor 2-dependent antioxidant response element activation by tert-butylhydroquinone and sulforaphane occurring preferentially in astrocytes conditions neurons against oxidative insult. J Neurosci 2004; 24:1101-12.
20. 20. Loreti S, Vilaro MT, Visentin S, Rees H, Levey AI, Tata AM: Rat Schwann cells express M1-M4 muscarinic receptor subtypes. J Neurosci Res 2006; 84:97-105.
21. 21. Cohen RI, Almazan G: Rat oligodendrocytes express muscarinic receptors coupled to phosphoinositide hydrolysis and adenylyl cyclase. Eur J Neurosci 1994; 6:1213-24.

22. 22. Chesler DA, McCutcheon JA, Reiss CS: Posttranscriptional regulation of neuronal nitric oxide synthase expression by IFN-gamma. J Interferon Cytokine Res 2004; 24:141-9.

Correspondence to:

Pamela J. Lein
Department of Molecular Biosciences
UC Davis School of Veterinary Medicine
One Shields Ave., 1120 Haring Hall
Davis, CA 95616
USA