Eosinophil-induced release of acetylcholine from differentiated cholinergic nerve cells

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Submitted 9 April 2003; accepted in final form 26 August 2003

Eosinophil-induced release of acetylcholine from differentiated cholinergic nerve cells. Am J Physiol Lung Cell Mol Physiol 285: L1296–L1304, 2003. First published August 29, 2003; 10.1152/ajplung.00107.2003.—One immunological component of asthma is believed to be the interaction of eosinophils with parasympathetic cholinergic nerves and a consequent inhibition of acetylcholine muscarinic M2 receptor activity, leading to enhanced acetylcholine release and bronchoconstriction. Here we have used an in vitro model of cholinergic nerve function, the human IMR32 cell line, to study this interaction. IMR32 cells, differentiated in culture for 7 days, expressed M2 receptors. Cells were radiolabeled with [3H]choline and electrically stimulated. The stimulation-induced release of acetylcholine was prevented by the removal of Ca2+. The muscarinic M2 receptor agonist arecaidine reduced the release of acetylcholine after stimulation (to 82 ± 2% of control at 10−7 M), and the M2 receptor antagonist AF-DX 116 increased it (to 175 ± 23% of control at 10−5 M), indicating the presence of a functional M2 receptor that modulated acetylcholine release. When human eosinophils were added to IMR32 cells, they enhanced acetylcholine release by 36 ± 10%. This effect was prevented by inhibitors of adhesion of the eosinophils to the IMR32 cells. Pretreatment of IMR32 cells with 10 mM carbachol, to desensitize acetylcholine receptors, prevented the potentiation of acetylcholine release by eosinophils or AF-DX 116. Acetylcholine release was similarly potentiated (by up to 45 ± 7%) by degranulation products from eosinophils that had been treated with N-formyl-methionyl-leucyl-phenylalanine or that had been in contact with IMR32 cells. Contact between eosinophils and IMR32 cells led to an initial increase in expression of M2 receptors, whereas prolonged exposure reduced M2 receptor expression.


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L1296 1040-0605/03 $5.00 Copyright © 2003 the American Physiological Society http://www.ajplung.org
adhesion molecules ICAM-1 and VCAM-1 and eosinophil integrins CD11/18 and very late antigen-4 (VLA-4). Adhesion leads to degranulation of the eosinophils and release of mediators, including EPO and leukotriene C4, and the generation of free radicals within the nerve cells. We have now used this model to investigate directly the effects of eosinophils on M2 receptors expressed in these cells (35) and how the various aspects of eosinophil-nerve cell interactions influence this process.

MATERIALS AND METHODS

Materials. All materials were purchased from Sigma-Aldrich (Poole, UK) unless otherwise indicated.

Culture of IMR32 human neuroblastoma cells. The human neuroblastoma cell line IMR32 (European Collection of Cell Cultures ECACC no. 86041809) was cultured in 25-ml flasks in proliferation medium consisting of DMEM (GIBCO-BRL, Paisley, UK) with 10% (vol/vol) FCS (GIBCO-BRL), 100 U/ml penicillin, 1 μg/ml streptomycin, and 100 μg/ml gentamicin, at 37°C in 5% CO2-95% air.

Gel electrophoresis and Western blotting to detect the M2 receptor. IMR32 cells were plated onto 24-well plastic plates at a density of 2 × 10⁴ cells/well and differentiated in differentiating medium [same as for proliferation medium above, but with 2% (vol/vol) FCS and 2 mM sodium butyrate] for between 3 and 7 days. The cells were lysed with 20 mM Tris (BDH-Merck, Poole, UK) containing 10 mM EDTA, 0.5% (vol/vol) Nonidet P-40, 0.5% (wt/vol) deoxycholate, 8 M urea, 2% (wt/vol) SDS, and 1% (wt/vol) bromphenol blue, pH 7.1, at a concentration of 2 × 10⁶ cells/ml. Samples were boiled for 5 min, and 2-mercaptoethanol was added to a final concentration of 5% (vol/vol). The samples were then separated on 8% SDS-polyacrylamide gels with a 4% polyacrylamide stacking gel, and the proteins were transferred to nitrocellulose paper (Hybond; Amersham Pharmacia Biotech, Little Chalfont, UK) as described previously (27).

The resulting Western blots were incubated with 5% (wt/vol) dried fat-free milk in Tris-buffered saline (TBS; 10 mM Tris and 140 mM NaCl, pH 7.4) for 1 h and then incubated overnight at 4°C with 1:10,000 dilution of mouse monoclonal anti-human M2 receptor antibody (31–1D1; Cambridge Bioscience, Cambridge, UK). Identical blots were incubated with mouse IgG1 immunoglobulin fraction (Caltag Laboratories, Silverstone, UK) at the same concentration to serve as control. Blots were then washed three times with 0.05% (vol/vol) dried fat-free milk in Tris-buffered saline (TBS; 10 mM Tris and 140 mM NaCl, pH 7.4) for 20 min at room temperature. The remaining granulocytes were washed in buffer consisting of 10% (wt/vol) PIPES (pH 7.4), 1% (wt/vol) glucose, and 2% (wt/vol) human serum albumin (Sigma, Munich, Germany). The remaining granulocytes were then incubated with anti-human CD16 microbeads (1 μl/10⁵ cells; Miltenyi Biotech, Bisley, UK) and an equal volume of buffer containing of 0.5% (wt/vol) BSA and 2 mM EDTA in PBS at 6°C for 45 min. The eosinophils were separated from contaminating neutrophils by the VarioMACS magnetic cell separation system (Miltenyi Biotech). Cell viability and purity were determined by trypan blue exclusion (ICN Biomedicals, Basingstoke, UK) and Diffiquick staining (Dade, Munich, Germany), respectively. Only those eosinophil preparations showing >95% viability and >98% purity were used.

RNA isolation and RT-PCR analysis of the M2 receptor. IMR32 cells were plated onto 24-well culture plates at a density of 2 × 10⁴ cells/well and differentiated for 7 days, as described above. Cells were then left untreated or were cocultured with 2 × 10⁵ eosinophils/well for 24 and 48 h. Total RNA was isolated from the cells with TRIzol reagent according to the manufacturer’s instructions. For quantitative LightCycler PCR (Roche Molecular Biochemicals, Lewes, UK), 1 μg of total RNA was reverse transcribed into cDNA with an oligo(dT)₁₅ primer by means of the First Strand cDNA Synthesis kit (Roche Molecular Biochemicals). Amplification of cDNA was carried out by quantitative PCR in a LightCycler in the presence of the double-stranded DNA binding dye SYBR Green 1 (Fast Start DNA Masters SYBR Green 1; Roche Molecular Biochemicals). Fluorescence was monitored during the PCR every 0.1°C. PCR mixtures contained 0.5 μM primers for either β-actin (sense: 5' TCC TGT GGC ATC CA TAC GAA ACT 3', antisense: 5' GAA GCA TTT GCG GTG GAC GAT 3') or for the human M2 receptor (sense: 5' GTG TGC AGC AAC GCC TCA GTT AT 3', antisense: 5' TCC CCA TCC TCC ACA GTT CTC 3'). The samples were denatured at 95°C for 10 min, followed by 45 cycles of annealing and extension at 95°C for 12 s, 55°C for 5 s, and 72°C for 10 s. The melting curves were obtained at the end of amplification by cooling the samples to 65°C for 15 s, followed by further cooling to 40°C for 30 s. Serial 10-fold dilutions were prepared from previously amplified PCR products of β-actin and M2, which were then used as standards to plot against the unknown samples. Data were quantified with LightCycler analysis software, and values were normalized to the level of β-actin expression for each sample on the same template cDNA.

Measurement of acetylcholine release from IMR32 cells. IMR32 cells were plated onto 24-well plates and differentiated for 7 days as described above. In some experiments, the
cells were pretreated with 10 mM carbachol for 1 h, with 0.1 mM carbachol for 24 h, or with 25 μM 2-(α-naphthyl)ethyltrimethylammonium iodide (α-NEHA) for 30 min. The cells were then incubated with [3H]methyl-choline chloride (74 KBq/ml; specific activity 20.8 GBq/mg choline; Amersham Pharmacia Biotech) in Trowell’s T8 (choline-free) medium (GIBCO-BRL) containing 5% (vol/vol) FCS, 100 U/ml (wt/vol) penicillin, 1 μg/ml (wt/vol) streptomycin, and 100 μg/ml (wt/vol) gentamicin. They were then further incubated in HEPES-buffered saline (HBS; 20 mM HEPES, 150 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl2, 0.8 mM MgSO4, and 25 mM glucose, pH 7.4) for 15 min and then in HBS containing 10 μM hemicholinium-3 for a further 15 min to inhibit reuptake of released choline. They were then again washed with HBS for 5 min.

At that point in the various experiments, IMR32 cells were incubated in Trowell’s medium alone, with various concentrations of the selective M2 receptor antagonist AF-DX 116 (Tocris Cookson, Bristol, UK) for 10 min, or with various concentrations of the nonselective muscarinic receptor agonist methacholine, the M2 receptor agonist arecaidine but-2-ynyl ester tosylate, with various numbers of human eosinophils in Trowell’s T8 medium for 30 min or with 10 mM carbachol for 1 h. In some cases, before the eosinophils were added to the nerve cells, the eosinophils were incubated for 30 min with 1 μg/ml of mouse anti-human monoclonal antibody to the M2 muscarinic receptor (a) or an isotype-matched IgG1 antibody (b). Immunoreactivity staining was detected in the isotype-matched controls (a); in contrast, no staining was detected in the isotype-specific immunoglobulin control (b). A: proteins from IMR32 cells that had been differentiated for between 3 and 7 days were extracted, separated by SDS-polyacrylamide gel electrophoresis, and blotted onto nitrocellulose. The Western blot was incubated with a mouse anti-human M2 muscarinic receptor antibody and subsequently with a peroxidase-conjugated goat anti-mouse antibody. Immunoreactivity was detected by chemiluminescence. Approximate molecular mass of the stained band is indicated. The blot is representative of 3 separate experiments.

Results

Expression and function of M2 muscarinic receptors on IMR32 cells. For the in vitro model to reflect the postulated mechanism of control of acetylcholine release by eosinophils, it was necessary to demonstrate that the differentiated IMR32 cells expressed M2 muscarinic receptors. Immunohistochemical analysis with a mouse anti-human monoclonal antibody to the M2 receptor (Fig. 1A) showed immunostaining on the cell bodies and along the neurites of the cells. Incubation with an isotype-matched control antibody showed no staining (Fig. 1A). This was confirmed by Western blotting, which indicated that the expression of the M2
muscarnic receptor by IMR32 cells increased during differentiation for up to 7 days (Fig. 1B).

M<sub>2</sub> receptor-regulated release of radiolabeled acetylcholine from IMR32 cells. The ability of differentiated IMR32 cells to release acetylcholine was assessed by radiolabeling the cells with [³H]methyl-choline chloride and then subjecting them to electrical field stimulation. IMR32 cells preincubated with [³H]choline released radioactivity into the medium; this basal release was mainly in the form of acetylcholine (Fig. 2B). This enhanced release of radioactivity was reproduced by exposing the cells to 53 mM KCl (Fig. 2B).

Stimulated release of radioactivity was reduced when cells were preincubated with the choline acetyltransferase inhibitor α-NEFA (25 μM) before uptake of [³H]choline, indicating that the released radioactivity was mainly in the form of acetylcholine (Fig. 2C). This was reinforced by the fact that the stimulation-induced release of radioactivity was inhibited if the experiments were performed in Ca²⁺-free medium in the presence of 2 mM EDTA (Fig. 2D).

The function of the M<sub>2</sub> muscarinic receptors expressed by IMR32 cells was examined by measurement of acetylcholine release after exposure to the M<sub>1</sub>/M<sub>2</sub> agonist arecaidine or the selective M<sub>2</sub> antagonist AF-DX 116. Both agents were effective close to their K<sub>d</sub> or K<sub>i</sub> values (1, 24). Pretreatment with the M<sub>2</sub> agonist arecaidine (Fig. 3A) inhibited the release of acetylcholine after stimulation. There was a similar decrease in acetylcholine release after treatment with the nonselective muscarinic receptor agonist methacholine (results not shown). Conversely, pretreatment with the selective M<sub>2</sub> antagonist AF-DX 116 led to an increase in acetylcholine release (Fig. 3B). These data are consistent with the release of acetylcholine being under the control of the M<sub>2</sub> receptors.

Eosinophil-induced enhancement of acetylcholine release. To determine whether the release of acetylcholine from the IMR32 cells was influenced by the presence of eosinophils, we incubated the nerve cells with eosinophils for 30 min (the time at which adhesion was maximal) and again measured release of radioactivity after stimulation. As previously shown (20), there was an increase in acetylcholine release from IMR32 cells compared with cells incubated without eosinophils. For example, 2 × 10⁴ eosinophils/well (containing 2 × 10⁴ IMR32 cells) significantly increased the release to 136 ± 10% of control (Fig. 3C). The enhanced release of acetylcholine was not due to a general cytotoxicity of the eosinophils toward the nerve cells (data not shown).

Because it was our hypothesis that eosinophil-induced potentiation of acetylcholine release from nerves may be due to antagonism at M<sub>2</sub> muscarinic receptors, we examined the effects of eosinophils on acetylcholine release under conditions in which the muscarinic receptors should be desensitized. In these experiments, the IMR32 cells were exposed to a high concentration (10 mM) of the nonselective muscarinic receptor agonist carbachol for 1 h before their exposure to eosinophils. Pretreatment with carbachol in itself reduced acetylcholine release after stimulation to 72 ± 4.4% of control. Consistent with a desensitization of the M<sub>2</sub> receptor, carbachol pretreatment completely prevented both the AF-DX 116-mediated enhancement of acetylcholine release and also prevented the stimulation by eosinophils (Fig. 4A).

Adhesion of eosinophils to IMR32 cells occurs through CD11/18 and VLA-4 integrins on the eosinophils. To determine the role of adhesion in the ability of eosinophils to enhance acetylcholine release, eosinophils were pretreated with a monoclonal blocking antibody to CD11/18 or with the VLA-4 peptide inhibitor L1299EOSINOPHILS AND ACETYLCHOLINE RELEASE

Fig. 2. A: IMR32 cells that had been differentiated for 7 days in 24-well culture plates were radiolabeled with [³H]methyl-choline chloride as described in MATERIALS AND METHODS. The cells were then electrically field stimulated for 1 min with a 2-ms pulse width at 5 V and 1 Hz. Cell-free samples were taken from each well before and after stimulation and analyzed for released radioactivity. Data are means ± SE of 7 separate experiments (*P < 0.02). B: cells were exposed for 10 min to normal Trowell’s medium or to Trowell’s medium containing elevated K<sup>+</sup> (53 mM KCl). Data are means ± SE of 3 separate experiments (*P < 0.03). C: cells were radiolabeled with [³H]methyl-choline chloride in the presence or absence of 25 μM 2-(α-naphthyl)-ethyltrimethylammonium iodide (α-NEFA), an inhibitor of choline acetyltransferase. Cells were then electrically stimulated for 1 min as described above. Data are means ± SE of 4 separate experiments (*P < 0.02). D: cells were radiolabeled with [³H]methyl-choline chloride and were incubated in either normal Trowell’s medium or in Ca²⁺-free Trowell’s medium containing 2 mM EDTA before being electrically stimulated. Data are means ± SE of 3 separate experiments (*P < 0.01).
ZD-7349 (17), neither of which had an effect on its own on acetylcholine release. The eosinophil-induced increase in acetylcholine release was inhibited after pretreatment with either ZD-7349 or the anti-CD11/18 antibody (Fig. 4).

Potentiation of acetylcholine release by factors released from eosinophils. Adhesion of eosinophils to IMR32 nerve cells leads to degranulation (20). To determine whether the effect of eosinophils on acetylcholine release was due to factors released on contact with IMR32 cells, eosinophils were stimulated with FMLP (10 μM), which also induces them to degranulate, or were preincubated with IMR32 cells, and the media from those eosinophils were added to IMR32 cells. Both sets of media produced a significant increase in acetylcholine release from IMR32 cells after stimulation, compared with untreated controls. In contrast, the medium from untreated eosinophils had only a small, statistically insignificant, effect on acetylcholine release (Fig. 4C).

Effect of eosinophils on M2 receptor expression. Finally, to determine the long-term effects of eosinophils on M2 receptor-related cholinergic function, we incubated eosinophils with IMR32 cells for various times up to 48 h, and the expression of M2 receptors was analyzed by quantitative PCR. There was an initial rise in the level of M2 muscarinic receptor mRNA expression in IMR32 cells after coculture with eosinophils at 24 h, but this fell to below baseline after 48 h (Fig. 5). Control experiments (data not shown) demonstrated that the eosinophils did not express detectable mRNA for M2 muscarinic receptors.

DISCUSSION

Previous work from our laboratory has shown that the IMR32 neuroblastoma cell line displays a phenotype in common with primary parasympathetic nerves, with expression of cell adhesion molecules ICAM-1 and VCAM-1 (29). These cell adhesion molecules, not necessarily exclusively, mediate the adhesion of human eosinophils to the cells (29), reflecting the known association between eosinophils and airway parasympathetic nerves in antigen-challenged guinea pigs and in human asthma (6). It was the purpose of this study to determine whether the interaction between human eosinophils and IMR32 cells would also simulate the postulated action of eosinophil degranulation products.

Fig. 3. A: IMR32 cells that had been differentiated for 7 days were radiolabeled with [3H]-methyl-choline chloride. The cells were incubated in either normal medium or in medium containing various concentrations of the M2 receptor agonist arecaidine (A), the selective M2 receptor antagonist AF-DX 116 (B), or various numbers of eosinophils (C). Cells were then electrically stimulated, and cell-free samples were taken from each well and analyzed for released radioactivity. Data are mean released radioactivity expressed as a percentage of that released from control cells in the absence of drugs ± SE of 3 separate experiments or 5 separate experiments (C). *P < 0.05; **P < 0.001.
to promote acetylcholine release through an effect at regulatory muscarinic M2 receptors (5, 10).

After differentiation for 7 days, IMR32 cells displayed a network of interconnecting neurites (29) and thus had the potential for engaging in at least some aspects of synaptic transmission. First, we demonstrated that the IMR32 cells were capable of taking up radiolabeled choline and that they released the radiolabel in response to electrical stimulation or a solution of high K+ (H11001). The electrically induced release was less than that produced by potassium, probably because not all the cells in a well would have received the maximal effect of the field stimulation. Release was not simply a result of damage to the cells, as it was abolished in Ca2+ (H9251)-free medium. This fact, along with the ability of -NETA to significantly inhibit the release at a concentration close to its in vitro EC50 value for inhibition of choline acetyltransferase (28), indicated that the release of radiolabeled material reflected nerve terminal acetylcholine release.

Three pieces of evidence, immunohistochemistry, Western blotting, and PCR, indicated that the cells expressed the acetylcholine M2 receptor, which is known to regulate the release of acetylcholine (16). Consistent with the receptor being functional, the muscarinic receptor agonist arecaidine, which acts at both M1 and M2 receptors (23), inhibited acetylcholine release. The agent has some selectivity for cardiac M2 receptors, but its precise degree of selectivity for M2 relative to M1 in IMR32 cells has not been determined.

The amount of inhibition of acetylcholine release (~20%) was broadly similar to that observed with guinea pig tracheal sections, in which it was used for the same purpose (32). Conversely, the selective M2
receptor antagonist AF-DX 116 (26) potentiated acetylcholine release by ~75%.

When eosinophils were added to the IMR32 cells, they potentiated acetylcholine release from the IMR32 cells. The maximum enhancement was somewhat less than that produced by the M2 receptor antagonist AF-DX 116 and was, therefore, within the range that could be explained by an effect on M2 receptors. Inhibitors of eosinophil adhesion to the nerve cells prevented this enhanced release; either an inhibitor of CD11/18/ICAM-1 interactions or of VLA-4/VCAM-1 interactions was sufficient to prevent the enhanced acetylcholine release. This is consistent with our previous findings that inhibiting either interaction reduced adhesion and that both sets of adhesion molecules/integrins are involved (29), possibly acting in synergy (4).

We then sought further evidence that the enhanced acetylcholine release was mediated by the M2 receptor. The M2 receptor can be internalized by exposure to a high concentration of the muscarinic receptor agonist carbachol (22). When we exposed IMR32 cells to 10 mM carbachol for 1 h, eosinophils no longer enhanced acetylcholine release. Muscarinic receptors can be down-regulated by carbachol, but the time course is likely to be of the order of several hours (31), and it was not possible to pretreat radiolabeled IMR32 cells with carbachol for that period of time without their losing the radiolabeled acetylcholine. The results were also complicated by the fact that carbachol itself reduced the release of acetylcholine. Although carbachol was washed from the medium before stimulation, it may not have been possible to reduce its levels sufficiently to prevent its acting as an agonist on the M2 receptor. However, importantly, carbachol pretreatment also prevented the acetylcholine receptor antagonist AF-DX 116 from enhancing acetylcholine release. Thus, carbachol, whether through its supposed action to internalize the receptor or by another means, had the same effect on the eosinophils as it did on a known M2 receptor antagonist. This points to the eosinophils potentiating acetylcholine release through an action on the M2 receptor.

Acetylcholine release could also be increased by application to the nerves of medium from eosinophils that had been in contact with another sample of IMR32 nerve cells or had been treated with FMLP. The amount of acetylcholine released by this medium was quantitatively similar to that produced by direct contact between similar amounts of IMR32 cells and eosinophils. This would suggest that the ability of eosinophils to enhance acetylcholine release was entirely due to their degranulation and release of active chemical mediators. Under the experimental conditions we employed, eosinophils, after exposure to either FMLP or IMR32 nerve cells, released both EPO and leukotriene C4 (20). As mentioned above, the enhancement of acetylcholine release caused by contact between eosinophils and IMR32 cells was completely abolished by treatment of eosinophils with either antibody to CD11/18 or the VLA-4 inhibitor ZD-7349, both of which reduce adhesion of eosinophils to IMR32 cells, the latter down to baseline levels (29). Those same treatments, however, inhibited the IMR32-induced release of EPO and LTC4 from the eosinophils by only ~50% (20). This apparent discrepancy can be interpreted in a number of ways. It is possible that the release of EPO and leukotriene C4 from eosinophils was not fully representative of the release of all products and that the release of other products would be completely inhibited by anti-CD11/18 antibody or ZD-7349. Alternatively, the enhanced release of acetylcholine produced by contact between eosinophils and IMR32 cells was a separate phenomenon from the enhanced release induced by the eosinophil degranulation products, and there was a threshold of effect of degranulation products that was not achieved in the presence of the inhibitors. At present, we are unable to distinguish between those two possibilities.

The data do indicate, however, that eosinophil products, whether wholly or partly, contribute to an increased release of acetylcholine from nerves. The most likely candidates for this effect are EPO and MBP, both of which are antagonists at M2 muscarinic receptors (18), with good supporting evidence from in vivo experiments for a major role for MBP (8). Currently, there is only circumstantial evidence that MBP mediates the action of eosinophils in this in vitro model. Heparin, which can bind to and inactivate MBP and can restore M2 receptor function in vivo, was able to prevent the effects of eosinophils to enhance acetylcholine release (results not shown); however, at the concentration used, heparin was also able to prevent adhesion of eosinophils to nerves (29). A more selective inhibitor, if available, would determine whether MBP is the sole molecule mediating the effect.

How specific was the action of eosinophils to promote acetylcholine release? The adhesion of eosinophils to IMR32 cells was not, in fact, selective: human neutrophils also adhered to the cells to a similar extent. However, most importantly, neither neutrophils nor the medium from neutrophils that had been incubated with a separate sample of IMR32 cells were able to alter acetylcholine release (results not shown).

The action of eosinophils on nerves, as simulated in this in vitro model, appears to have both an acute and a chronic component. The acute effect of contact with eosinophils is associated with a number of downstream events in the IMR32 cells that include the production of reactive oxygen species (21), protein tyrosine phosphorylation, induction of transcription factors (34), and remodeling of nerve morphology (21). These phenomena are, at least in part, a consequence of cell adhesion molecule interactions. The M2 muscarinic receptor is a G protein-coupled receptor linked to inhibition of adenylyl cyclase (19) and to the Egr family of transcription factors (33). Eosinophils and eosinophil-derived factors could interact at either of those sites to alter protein expression. The data presented here indicate that in addition to the acute effect of eosinophil-mediated loss of M2 receptor function, prolonged exposure leads to an initial upregulation of the M2 receptor itself and a later reduction in the M2 receptor mRNA. In
other studies (21), we have shown that prolonged exposure to eosinophils is not associated with cell death nor apoptosis; thus, it unlikely that this reduction was due to a cell death. The significance of this finding may be that prolonged exposure to eosinophils may lead to loss of function of M₂ receptors by a mechanism separate from the direct effect of eosinophils on the function of the receptor. Now that the human M₂ promoter has been identified (35), it should be possible to determine the precise link between eosinophil interactions and the changes we observed in M₂ receptor expression.

In summary, we have produced a model that simulates the effects of eosinophil/nerve interactions on cholinergic nerve function and confirmed the hypothesis that interactions between nerves and eosinophils can modulate both acetylcholine release and M₂ receptor expression. The model is currently being used to determine the molecular mechanisms underlying both of these processes.

We thank Emma Court for technical assistance.

DISCLOSURES

This work was supported by Wellcome Trust (063859) and a Samuel Crossley-Barnes research studentship (to D. A. Sawatzky).

REFERENCES


31. Steel MC and Buckley NJ. Differential regulation of muscarinic receptor mRNA levels in neuroblastoma cells by chronic...


