Subcellular Distribution of Muscarinic Acetylcholine Receptors in Rat Exorbital Lacrimal Gland

Michael E. Bradley, Chris L. Peters, Ross W. Lambert, Samuel C. Yiu, and Austin K. Mircheff

The muscarinic acetylcholine receptor (MACHR) is an important mediator of parasympathetic regulation of secretion by the rat exorbital lacrimal gland. In order to survey the subcellular distribution of MACHR in lacrimal acinar cells, we have measured the binding of the specific muscarinic cholinergic antagonist [3H]-quinuclidinyl benzilate ([3H]-QNB) to membrane samples isolated from rat exorbital lacrimal glands by differential and equilibrium density gradient centrifugation. Binding of [3H]-QNB in all membrane fractions was consistent with the presence of a single class of receptor which was muscarinic in nature on the basis of its K^ for [3H]-QNB (0.30-0.35 nM) and its ability to interact with the muscarinic agonists carbachol and methachol and the antagonist atropine. MACHR were present at the highest specific activity in acinar cell basal-lateral plasma membrane-derived populations, where B_{max} was as high as 1960 fmole/mg protein. However, the density distributions of MACHR and of other membrane markers indicated that the receptors were present also in membranes derived from cytoplasmic structures, where B_{max} values ranged from 50.4 to 152.8 fmole/mg protein. Stimulation with 10 μM carbachol for 30 min led to a 20% (P < 0.05) increase in the relative MACHR content of a population of membranes derived from the acinar cell basal-lateral membrane; an apparent tendency for MACHR activity to decrease in other membrane populations suggests that stimulation might cause a redistribution of MACHR between cytoplasmic pools and the cell surface membranes. Invest Ophthalmol Vis Sci 31:977-986, 1990

Muscarinic acetylcholine receptors (MACHR) are, at least in part, responsible for mediating the parasympathetic stimulation of fluid, electrolyte, and macromolecular product secretion by the lacrimal glands.1-3 The receptors are understood to reside in the lacrimal acinar cell basal-lateral surface membrane, and binding of agonists to the receptors initiates a sequence of events which culminates in the activation of a number of different functions. These functions include the exocytic fusion of secretory vesicles with the apical membranes, conductive efflux of Cl^- through the apical membranes,4 conductive efflux of K^+ through the basal-lateral membranes,5 and coupled influxes of Na^+ and Cl^- through the basal-lateral membranes.6,7

From the Department of Physiology and Biophysics and Department of Ophthalmology, University of Southern California School of Medicine, Los Angeles, California.

Supported by National Institutes of Health Grants EY-05711 and EY-05801 and by a grant from the University of Southern California Faculty Research and Innovation Fund. MEB was a trainee of National Institutes of Health Grant GM-08017. MEB, RWL, and SCY were recipients of Sigma Xi Grants-in-Aid of Research.

Submitted for publication: May 10, 1989; accepted September 13, 1989.

Reprint requests: Austin K. Mircheff, PhD, Department of Physiology and Biophysics, University of Southern California, School of Medicine, 1333 San Pablo Street, Los Angeles, CA 90033.

Several of the enzymes and transporters that are expressed in the plasma membranes of the lacrimal acinar cell are present also in substantial cytoplasmic pools,8-11 which have been resolved by subcellular fractionation analyses. According to current concepts of membrane biogenesis, such pools are expected to include newly synthesized proteins en route through the membrane assembly pathway, and perhaps also to include internalized proteins destined to be either recycled or degraded. However, it is becoming apparent that certain cytoplasmic pools of plasma membrane-expressed proteins participate in important ways in electrolyte secretion mechanisms. A Golgi membrane-associated pool of one important basal-lateral membrane protein, Na,K-ATPase, functions as a reserve available for rapid mobilization and recruitment to the basal-lateral membrane following MACHR activation.12 In other exocrine glands, it appears that Cl^- channel proteins are associated with the secretory granule membranes of resting acinar cells and are inserted into the apical plasma membranes upon stimulation.13 In the parietal cell of the gastric glands, proton-translocating ATPases are inserted into and retrieved from the apical surface in the transitions between stimulated and resting states.14

It is not known whether lacrimal acinar cells contain cytoplasmic pools of MACHR comparable to
those demonstrated for other basal-lateral mem-
brane-expressed proteins. This question seemed par-
ticularly interesting in light of recent studies of the
dynamic behavior of MACHR in several different cell
types. In resting astrocytoma cells, neuroblastoma
cells, and embryonic cardiac myocytes, MACHR appear
to be confined to the surface membranes, but a
rapid endocytic internalization of receptors de-
creases surface-expressed MACHR activity by 50% or
more during a 30-min exposure to the agonist, carba-
chol. MACHR down-regulation follows a much
slower time course in pancreatic acinar cells, which in
many ways function analogously to lacrimal acinar
cells. Whereas overnight culture of pancreatic acini in
the presence of carbachol decreases surface-expressed
MACHR activity by 90%, receptor activation for 30
min leads to only a 15–20% decrease of activity.18

In the current study, we have characterized the
binding of the potent muscarinic cholinergic antago-
nist, L-[3H]-quinuclidinyl benzilate ([3H]-QNB) to
plasma and cytoplasmic membrane samples isolated
from rat exorbital lacrimal glands. We have found
that MACHR have their highest specific activities in
binding of the potent muscarinic cholinergic antago-
nist, L-[3H]-quinuclidinyl benzilate ([3H]-QNB) to
plasma and cytoplasmic membrane samples isolated
from rat exorbital lacrimal glands. We have found
that MACHR have their highest specific activities in
the basal-lateral plasma membranes, but that they
are also present in several cytoplasmic membrane
populations. The cytoplasmic populations together
account for roughly half of the total recovered ligand
binding activity, and they have the same [3H]-QNB
affinities and sensitivities to competing ligands as the
plasma membrane-associated sites. Stimulation with
carbachol for 30 min increases the MACHR activity
of a distinct basal-lateral membrane-derived popula-
tion, possibly by causing MACHR to be recruited
from cytoplasmic membrane-associated pools.

**Materials and Methods**

**Preparation of Tissue**

All experiments were performed in accordance
with the ARVO Resolution on the Use of Animals in
Research. The details of tissue preparation and cell fractionation have been described previously.1,12 For
each experiment, eight male Sprague-Dawley rats
(240–260 g) were killed by cervical dislocation. Exor-
bital lacrimal glands were immediately dissected free
of connective tissue and placed into a modified Krebs
improved Ringer’s I bicarbonate buffer (KRB) in
which beta-hydroxybutyrate replaced glucose, fuma-
rate, glutamate, and pyruvate, and to which 0.2 mM
phenylmethylsulfonyl-fluoride (PMSF) and 9 µg/ml
aprotinin were added as protease inhibitors. KRB
was kept at 37°C and gassed continuously with 95%
O2/5% CO2. The baskets were transferred to fresh aliquots of
KRB at 5-min intervals. In most experiments, the
total incubation time was 55 min, since peroxidase
release, an indicator of lacrimal secretion, reaches a
stable baseline value within this equilibration pe-
riod.12 In experiments designed to test the effect of
stimulation on the MACHR subcellular distribution,
icubations were allowed to continue for additional
periods of 30 min in the presence and absence of 10
µM carbachol.12

**Subcellular Fractionation**

At the end of the in vitro incubation period, the
tissue fragments were rinsed rapidly with ice-cold
isolation buffer and transferred to a large glass tube
containing 18 ml isolation buffer. Isolation buffer
consisted of 5% sorbitol, 0.5 mM EDTA, and 5 mM
histidine-imidazole buffer, pH 7.5; isolation buffer
and all density gradient media contained 0.2 mM
PMSF and 9 µg/ml aprotinin.10–12 The tube was
placed in an ice-water bath, and the fragments were
homogenized with a Tissumizer® (Tekmar Instru-
ments, Cincinnati, OH) for 20 min. The homogenate
was centrifuged at 2000 g for 10 min. The superna-
tant was withdrawn, and the pellet was subjected to
three cycles of homogenization for 10 min and cen-
trifugation at 2000 g for 10 min. The pooled super-
natants then were brought to a final sorbitol concen-
tration of approximately 55% and subjected to cen-
trifugation at 235,000 g for 60 min in a Beckman Z-60
zonal rotor loaded with a two-stage (35–55% and
55–70%) sorbitol gradient.9,19 At the conclusion
of the centrifugation run, the rotor was unloaded by
displacement with 80% sucrose, and its contents were
collected in 24 equal fractions. The fractions were
diluted 1.8-fold with isolation buffer and centrifuged
at 250,000 g for 75 min; the resulting pellets were
resuspended in 2.4-ml aliquots of isolation buffer.
Aliquots (1.8 ml) were removed, quickly frozen in
liquid nitrogen, and stored at −70°C until used for
binding experiments. The remaining material was
kept at 4°C until the following day, when biochemical
markers were determined according to procedures
described previously.19

**MACHR Determinations**

Density gradient fractions were pooled into six
density windows on the basis of particular features of
the biochemical marker distribution patterns. Sam-
ple density windows on the basis of particular features of
the biochemical marker distribution patterns. Sam-
ple
mM sodium phosphate, and 0.2 mM PMSF, pH 7.4). After centrifugation at 250,000 g for 60 min, the resulting pellets were suspended in phosphate-buffered sorbitol without PMSF.

Binding reactions were performed at 25°C with constant shaking in a temperature-controlled water bath; the total reaction volume was 1.2 ml. Unless otherwise indicated, the reaction medium contained phosphate-buffered sorbitol (pH 7.4), 2.25 nM [3H]-QNB, and 0.1–0.6 μg membrane protein. Samples were preincubated for 60 min at 25°C in the presence or absence of 2.5 μM atropine, or, in some experiments, 0.1 μM unlabeled QNB. The reaction was then allowed to continue for 60 min after addition of [3H]-QNB. Reactions were quenched by addition of 3 ml ice-cold phosphate-buffered sorbitol. Quenched reactions were immediately filtered through Whatman GF/B filters in a Brandel M-12 Manifold Cell Harvester® (Biomedical Research and Development Laboratories, Gaithersburg, MD) and washed three times with 4-ml aliquots of ice-cold phosphate-buffered sorbitol. Filters were placed in 8 ml Filtron-X® scintillation fluid (National Diagnostics) and extracted by shaking for 60 min. Counting was performed in a Beckman LS-8000 liquid scintillation counter. Specific binding was calculated as the component displaced by 2.5 μM atropine or 0.1 μM QNB. Preliminary experiments confirmed that—as has been found for MACHr in other tissues—[3H]-QNB binding was substantially complete by 60 min; the time course of [3H]-QNB binding was not altered by treatment with digitonin, indicating that membrane permeation was not rate-limiting; furthermore, binding increased linearly with increasing sample protein.

Materials

L-quinicinuliny-phenyl-4-3H-benzilate was obtained from Amersham (Arlington Heights, IL). Carbamylcholine chloride (carbachol), acetyl-beta-methylcholine chloride (methachol) and atropine sulfate were obtained from Sigma (St. Louis, MO). Unlabeled QNB was the generous gift of Dr. Steven Flanagan. Analyzed reagent grade D-sorbitol was from J. T. Baker (Phillipsburg, New Jersey). All other chemicals were reagent-grade and were obtained from standard suppliers.

Results

Subcellular Fractionation

The density distributions of biochemical markers, depicted in Figure 1, were similar to those obtained when lacrimal gland scrapings were subjected to subcellular fractionation without an in vitro equilibra-

Density Distribution of MACHr

Figure 2 summarizes the density distribution of [3H]-QNB binding activity. The peak of specific [3H]-QNB binding coincided with the peaks of Na,K-ATPase and alkaline phosphatase in window II (Figure 1). The overall distribution of MACHr activity was similar to the distribution of alkaline phosphatase and to the previously reported distributions of Na/H antiport activity, Cl/HCO3 antiport activity, and several different amino acid transport activities.
brane-expressed markers all differed from that of Na,K-ATPase in that they lacked clearly defined peaks in window IV. As noted in the legend to Figure 2, we can estimate that MACHR activity was enriched 18-fold in window I and 16.8-fold in window II. Since the Na,K-ATPase cumulative enrichment factors were 15.1 in window I and 11.2 in window II, it appears that MACHR activity is a somewhat more specific marker for basal-lateral membranes than is Na,K-ATPase.

Of the total [3H]-QNB binding activity recovered from the density gradient, 20% was present in window I; 29% was present in window II; and 51% was present in windows III–VI. Comparison of the MACHR and succinate dehydrogenase distributions suggests that little of the MACHR activity was associated with the mitochondria. Unfortunately, MACHR activity was lost during phase partitioning analyses, so it was not possible to determine how the receptors were distributed among the endoplasmic reticulum, Golgi, and miscellaneous minor membrane populations which equilibrated in windows III–VI. Although the precise subcellular distribution of MACHR could not be determined, it was of inter-

Fig. 1. Density distributions of biochemical markers from a lacrimal gland fragment preparation typical of those used in the experiments described in Figures 2–5 and Table 1. Fragments were incubated in vitro for 55 min, and cell fractionation and marker determinations were performed as described under Materials and Methods. Activity recovered in each density window is divided by the number of fractions in the window and is expressed as a percentage of the total activity recovered in the high-speed pellets generated from the 24 density gradient fractions. The ratio of marker activity to protein in these units indicates the incremental enrichment of marker produced by the density gradient separation. Since the high-speed pellets accounted for 69% ± 3% of the Na,K-ATPase and 30.9% ± 1.4% of the protein recovered from the initial homogenate, the cumulative enrichment factors for Na,K-ATPase in windows I–VI were 15.1, 11.2, 2.3, 1.5, 0.9, and 0.9.
were similar to those associated with basal-lateral to 0.971. These results indicate that a single class of protein in window I.

concentration-dependent of specific binding became maximal at concentrations of 0.31-0.35 nM, and regression coefficients from the linear over the range of concentrations studied, specificity of the cholinergic receptor ligands carbachol, methachol, and atropine to prevent [3H]-QNB binding. The resulting displacement curves, which are illustrated in Figure 4, indicated that displacement was half-maximal between carbachol concentrations of 0.4 mM and 1.0 mM, between methachol concentrations of 0.1 mM and 1.0 mM, and between atropine concentrations of 5 nM and 10 nM. There were no consistent differences between the specificities of the MACHR activities in the plasma membrane-associated and cytoplasmic membrane-associated pools.

Inhibition of [3H]-QNB Binding by Other Ligands

Pharmacologic specificity of the [3H]-QNB binding in windows I–IV was assessed by measurement of the abilities of the cholinergic receptor ligands carbachol, methachol, and atropine to prevent [3H]-QNB binding. The cumulative enrichment factors calculated in this way for windows I-VI are 18.0, 16.9, 2.9, 1.3, 0.4, and 0.2.

est to compare characteristics of the MACHR activities in the basal–lateral membrane-containing and cytoplasmic membrane-containing fractions.

Concentration-Dependence of Specific [3H]-QNB Binding

[3H]-QNB binding isotherms were constructed for samples from density windows I–V in the presence and absence of 0.1 μM unlabeled QNB; these isotherms and their Scatchard transformations are presented in Figure 3. While nonspecific binding was linear over the range of concentrations studied, specific binding became maximal at concentrations of 2.0 nM or less. The Kd values, maximal binding levels, and regression coefficients calculated from the Scatchard transformations are summarized in Table 1. Kd values varied within the narrow range of 0.31–0.35 nM, and regression coefficients from the Scatchard transformations were greater than or equal to 0.971. These results indicate that a single class of receptor was present in each window and that the receptors associated with cytoplasmic membranes were similar to those associated with basal–lateral plasma membranes. On the other hand, the Bmax values varied through a 40-fold range, from 50 fmole/mg protein in window V to 1960 fmole/mg protein in window I.

Fig. 2. Density distribution of specific [3H]-QNB binding. As in Figure 1, the total specific binding in each density window has been divided by the number of fractions in the window and expressed as percentage of total recovered specific binding activity. Values are means ± SD for four separate lacrimal gland fragment preparations which had been incubated in vitro for 55 min. Although MACHR was not measured in the initial homogenate, low-speed pellet, low-speed supernatant, or high-speed supernatant, it is possible to estimate the fraction of the cell's total MACHR, and therefore the MACHR cumulative enrichment, by assuming that MACHR distributed in parallel with Na,K-ATPase through the separation steps leading up to the high-speed pellets from the density gradient fractions. The cumulative enrichment factors calculated in this way for windows I–VI are 18.0, 16.9, 2.9, 1.3, 0.4, and 0.2.

The possibility that receptor activation might lead to a change in the disposition of MACHR activity between the basal–lateral membrane-associated and cytoplasmic membrane-associated pools was assessed by subcellular fractionation analyses of resting and carbachol-stimulated lacrimal gland fragments. Fragments to be stimulated were incubated in KRB containing 10 μM carbachol for an additional 30 min after the 55-min equilibration period routinely used in the experiments described above. Control (resting) lacrimal gland fragments were incubated in KRB for 85 min. As has been reported by Yiu et al., this stimulation interval is accompanied by significant changes in the distributions of Na,K-ATPase and acid phosphatase, whereas the distributions of alkaline phosphatase, NADPH–cytochrome c reductase, succinate dehydrogenase, and protein remain unchanged. Na,K-ATPase pump units appear to be translocated from density windows IV and V to density window I, increasing the Na,K-ATPase activity of window I by 40%. Acid phosphatase appears to be removed from a population of very slowly sedimenting vesicles not represented in the density gradient fractions and inserted into membranes equilibrating in density windows I, II, and III. Stimulation for 30 min has no effect on the total activity measured for any of the enzymatic markers.

The difference between the density gradient distributions of MACHR from resting and stimulated preparations, depicted in Figure 5, indicates that stimulation increased the relative yield of MACHR activity in density window I by 20%. The MACHR activities of density windows II–VI appeared to decrease after stimulation, although these changes were not statistically significant. Total MACHR activity recovered from windows I–VI from resting preparations was 141 ± 42 fmole/mg membrane protein; the corresponding value for stimulated preparations was 114...
Fig. 3. Concentration dependence of \([3^H]\)-QNB binding. Specific binding as a function of \([3^H]\)-QNB concentration was measured with samples from density windows I–V. Each point is the mean of triplicate determinations from the same lacrimal gland fragment preparation. Also presented are Scatchard transformations of the resulting data. \(K_d\) and \(B_{max}\) values are summarized in Table 1.

\[\pm 15 \text{ fmole/mg membrane protein (} P = 0.18\). These values suggest that, in addition to causing a redistribution of MAChR to the membranes equilibrating in density window I, 30-min stimulation with carbachol might slightly decrease the cell's total MAChR activity.

Discussion

The characteristics of MAChR have been studied with whole cell, cell lysate, and crude membrane preparations from a variety of tissues and cell lines.1,2,22–27 Such studies have elucidated MAChR ligand-binding properties, distinguished receptor subtypes, and permitted estimates of receptor masses. The receptor characteristics in lacrimal acinar cells outlined in the current study are similar to those reported for other cell types.

To our knowledge, this is the first systematic survey of the subcellular distribution of MAChR in an epithelial cell type. The question of the MAChR subcellular distribution has received more attention in the excitable cell types which rapidly internalize activated MAChR. Two different kinds of experiment have led to the conclusion that, prior to activation, MAChR are confined largely to the plasma membranes of excitable cells. First, measurements of the abilities of intact cardiac myocytes17 and neuroblastoma cells16 to bind \([3^H]\)-QNB, a relatively lipophilic muscarinic ligand, and \([3^H]\)-NMS, a relatively hydrophilic muscarinic ligand, yielded similar numbers of binding sites for the two different ligands. Second, in subcellular fractionation analyses of resting astrocytoma cells which had been treated with Con A to increase the plasma membrane equilibrium density, the distribution of MAChR activity on sucrose gradients was similar to the distribution of adenyl cyclase, a plasma membrane marker.15 Although these lines of evidence are strong, it should be noted that the first type of measurement does not exclude the
possible presence of receptors which recycle between the plasma membranes and cytoplasmic compartments over the 60- to 90-min time courses of the binding reactions, and that the second type of experiment does not address the possible existence of binding sites on cytoplasmic membranes which equilibrate at the same densities as the plasma membranes.15

Comparison of the MACHR density distribution in Figure 2 with the marker density distributions in Figure 1 indicates that MACHR activity is divided evenly between surface membrane-expressed and cytoplasmic membrane pools in lacrimal acinar cells. Approximately half of the recovered MACHR activity is associated with membranes equilibrating in density windows I and II. The membranes in window I can be identified unambiguously as basal-lateral plasma membranes 1) because they have the highest observed specific contents of such typical plasma membrane-expressed constituents as Na,K-ATPase, alkaline phosphatase, Na/H-antiporters,10 Cl/HCO3 antiporters,11 and now, MACHR, and 2) because they have relatively small contents of the cytoplasmic membrane markers succinate dehydrogenase, NADPH-cytochrome c reductase, and galactosyltransferase.8,10

Density window II contains three different membrane populations; these have been resolved by partitioning analyses in aqueous polymer two-phase systems, which distinguish membranes on the basis of differences in their surface properties.28 The most important component of window II is a population enriched in both Na,K-ATPase and alkaline phosphatase; it differs from the basal-lateral membrane population in window I in having slightly lower enrichment factors for the plasma membrane markers and a higher enrichment factor for galactosyltransferase.8,28 On the basis of these characteristics, this population appears to represent a basal-lateral membrane microdomain distinct from that represented in win-

![Fig. 4. Inhibition of [3H]-QNB binding in samples from density windows I-IV by muscarinic receptor ligands atropine (triangles), carbachol (circles), and methachol (squares). [3H]-QNB concentration was 2.25 nM. Each point is the mean of triplicate determinations with samples from the same lacrimal gland fragment preparation; similar results were obtained with samples from a second preparation.](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933153/ on 09/23/2017)
window I. However, we are unable to exclude the possibility that it might have been derived from a domain of the Golgi complex involved in basal-lateral plasma membrane assembly and recycling.8,12 Also represented in window II are a small cytoplasmic membrane marker activities observed in windows III-V, it seems reasonable to infer that their contents of Na,K-ATPase relative to the other plasma membrane marker activities. Second, while another plasma membrane marker, alkaline phosphatase, appears to have a unimodal, skewed density distribution when fractions are pooled into density windows (as in Fig. 1), a fraction-by-fraction presentation (eg, as in Fig. 2 of reference 12), can be visualized as the resultant of the contributions of a series of overlapping populations. Third, phase partitioning analyses of density windows III–V performed under a variety of separation conditions yielded consistent parallels between the distributions of virtually all of the Na,K-ATPase activity, the major component of the alkaline phosphatase activity, and the galactosyltransferase activity, indicating that all three markers were primarily associated with the same populations of membranes.8 Since the partitioning behavior of the basal-lateral membranes from window II differed markedly from that of the Golgi-derived membranes from window III, these analyses also confirmed that there was little spillover of basal-lateral membranes into window III (Bradley ME, manuscript in preparation).

Because of the association of Na,K-ATPase and alkaline phosphatase with Golgi-derived membranes in windows III–V, it seems reasonable to infer that the MACHr, as well as the Na/H and Cl/HCO3 antiport activities in these windows are localized primarily to Golgi-derived membranes. There are a number

<table>
<thead>
<tr>
<th>Window</th>
<th>Ke (nM)</th>
<th>Bmax (fmole/mg)</th>
<th>Regression coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.324</td>
<td>1960</td>
<td>0.971</td>
</tr>
<tr>
<td>II</td>
<td>0.332</td>
<td>1414</td>
<td>0.992</td>
</tr>
<tr>
<td>III</td>
<td>0.305</td>
<td>153</td>
<td>0.996</td>
</tr>
<tr>
<td>IV</td>
<td>0.308</td>
<td>74</td>
<td>0.977</td>
</tr>
<tr>
<td>V</td>
<td>0.346</td>
<td>50</td>
<td>0.985</td>
</tr>
</tbody>
</table>

Values were obtained by linear regression analyses of the Scatchard transformations presented in Figure 3. Note that different amounts of protein are present in each density window, so that the total number of binding sites per preparation is the weighted average of the above Bmax values.
of reports of large cytoplasmic membrane-associated pools of plasma membrane-expressed constituents in other cell types. For example, Tamkun and Fambrough have found that two thirds of the Na,K-ATPase in intact cultured chick myotubes was inaccessible to the cell surface; Carbonetto and Fambrough found approximately the same value for the cytoplasmic pool of alpha-bungarotoxin receptors in chick sympathetic neurons. Jacob and co-workers have documented a substantial cytoplasmic pool of nicotinic acetylcholine receptor immunoreactivity in embryonic chick ciliary ganglion neurons.

As noted in the introduction to the current report, a portion of the Golgi-associated Na,K-ATPase activity in lacrimal acinar cells functions as a reserve which is rapidly translocated to the basal-lateral membranes after stimulation with carbachol. The significance of the large cytoplasmic, presumably Golgi-associated, pools of MACHR and other basal-lateral membrane-expressed activities in the acinar cell is not entirely clear. A portion of the Golgi-associated activity of each marker is almost certainly attributable to newly synthesized peptides en route to the plasma membranes. It is possible that another portion of each Golgi-associated plasma membrane-expressed activity is attributable to the recycling of peptides which have been internalized from the lacrimal acinar cell plasma membranes. That the cytoplasmic pools of recycling plasma membrane constituents may be quite substantial is suggested by recent studies with HTC and HeLa cells, where the internalized pools account for two thirds of the cell content of surface-expressedialoglycopeptides. Finally, the stimulation-associated redistribution of MACHR activity depicted in Figure 5 raises the possibility that the cytoplasmic MACHR pool might also function as a reserve from which receptors can be mobilized and inserted into the basal-lateral membrane microdomain represented by the membrane population equilibrating in density window I. This microdomain is also the locus into which Na,K-ATPase pump units and, possibly, Cl/HCO₃ antiporters are inserted after cholinergic stimulation.

If it is true that stimulation leads MACHR to be translocated from the cytoplasmic pool to a dynamic basal-lateral membrane domain, one can predict that the relationship between MACHR translocation and Na,K-ATPase translocation is complex. This is because the Na,K-ATPase units are specifically mobilized from membranes which equilibrate in density windows IV and V, while the data in Figure 5 suggest that MACHR might be recruited from windows II–V. If MACHR activation accelerates the internalization of basal-lateral membrane constituents, as it does in pancreatic acinar cells, and if a fraction of the internalized MACHR are degraded, as in excitable cells, then the existence of a substantial recruitable pool of cytoplasmic MACHR could serve to buffer the plasma membrane-expressed MACHR activity to approximately constant levels during an initial period after stimulation. According to this tentative hypothesis, prolonged exposure to agonists could eventually deplete the cytoplasmic pool, leading to a gradual down-regulation of MACHR similar to that observed in pancreatic acini.

Key words: tear film, lacrimal secretion, stimulus-secretion coupling, plasma membranes, Golgi complex

Acknowledgments

We thank Dr. Steven D. Flanagan, Neurosciences Division, City of Hope National Medical Center, Duarte, California, for the generous gift of unlabeled QNB and for loaning the Bandel Harvester used in this study.

References