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Stimulation-Associated Redistribution of Na,K-ATPase in Rat Lacrimal Gland

Samuel C. Yiu, Ross W. Lambert, Michael E. Bradley, Claire E. Ingham, Klane L. Hales, Richard L. Wood,† and Austin K. Mircheff

Department of Physiology and Biophysics, and †Department of Anatomy and Cell Biology, University of Southern California, School of Medicine, Los Angeles, California 90033

Summary. To test the possibility that stimulation of secretion leads Na,K-ATPase to be recruited from cytoplasmic pools and inserted into basal-lateral plasma membranes, we surveyed the subcellular distributions of Na,K-ATPase in resting and stimulated fragments of rat exorbital lacrimal gland. After a two-dimensional separation procedure based on differential sedimentation and density gradient centrifugation, we defined six *density windows*, which differ from one another in their contents of biochemical markers. The membranes equilibrating in *window I* could be identified as a sample of basal-lateral membranes; in resting preparations these membranes contained Na,K-ATPase enriched 16.6-fold with respect to the initial homogenates. *Windows II* through *VI* contained various cytoplasmic membrane populations; these accounted for roughly 80% of the total recovered Na,K-ATPase activity. Thirty-minute stimulation with 10 μM carbachol caused a 1.4-fold increase ($P < 0.05$) in the total Na,K-ATPase content of *window I*; this increase could be largely accounted for by a 1.7-fold decrease in the total Na,K-ATPase content of *density window V*. Acid phosphatase activity also redistributed following stimulation, but it was recruited from a different source, and it was inserted into membranes equilibrating in *windows II* and *III* as well as into the membranes of *window I*.

Key Words exocrine glands · fluid and electrolyte secretion · cell volume regulation · Golgi complex · membrane recycling · stimulus-secretion coupling

Introduction

The absorptive epithelia regulate their cytosolic sodium activities (a_{Na}) within narrow limits while generating transcellular Na^+ fluxes of rapidly and widely varying magnitudes. Since Schultz [31] first reviewed the evidence for homocellular regulation of a_{Na}, the phenomenon has been documented in small intestine [10], urinary bladder [14, 16], and gallbladder [22]. In contrast, the regulation of cytosolic a_{Na} in secretory epithelia has received little attention.

Fluid secretion in many mammalian epithelia depends on basal-lateral membrane-localized coupled Na^+-Cl^- transport processes which use the Na^+ electrochemical potential gradient, established and maintained by Na,K-ATPase, to accumulate Cl^- against electrochemical equilibrium. While Cl^- ions move from the cell to the lumenal compartment via conductance pathways localized in the apical membranes, most Na^+ ions are returned to the interstitial fluid by Na,K-ATPase pump units in the basal-lateral membranes. The Cl^--dependent transepithelial voltage then drives a secretory flux of Na^+ through the paracellular pathway [33].

Rates of fluid secretion are under neural or hormonal control, and they can vary through wide ranges [27]. The rat exorbital lacrimal gland is one relatively well-studied example. At rest, it produces fluid at a rate of 0.1 μl min^{-1}, and its secretory rate increases to 1.2 μl min^{-1} following cholinergic stimulation [1]. Given the secretory mechanism outlined above [3], the transcellular Cl^- flux must also increase 12-fold, and the Cl^--coupled Na^+ influx across the basal-lateral membrane should increase commensurately. In fact, estimates of initial rates from $^{22}Na^+$ uptake measurements [26] indicate that the flux of Na^+ into lacrimal acinar cells increases more than 10-fold as a consequence of stimulation with acetylcholine.

In order to regulate its volume and maintain the Na^+ gradients necessary for Na^+-coupled Cl^- accumulation, the secretory epithelial cell must counterbalance the stimulation-induced acceleration of Na^+ influx by rapidly increasing its Na,K-ATPase pump rate. It appears that the stimulation-induced acceleration of Na^+ influx leads to a measurable elevation of cytosolic a_{Na}. For example, a_{Na} has been reported to increase 1.5-fold in pancreatic acini [23] and nearly 2.0-fold in canine tracheal epithelium [32]. In mouse lacrimal acinar cells, a_{Na} increases 2.3-fold, from a resting level of approximately 6 mM

to a maximally stimulated level of 14 mM [30]. The kinetic properties of Na,K-ATPase are such that increases of a_{Na} within this range should lead to relatively large increases in the pump rate [16]. However, it seems likely that additional mechanisms also contribute to the lacrimal acinar cell's ability to rapidly accelerate its Na,K-ATPase pump rate.[1]

According to current hypotheses for short-term regulation of membrane transport processes, possible additional mechanisms for accelerating the pump rate might include activation of pump units already in place in the membrane and recruitment of additional pump units from preformed, cytoplasmic stores. Since analytical subcellular fractionation studies have already shown that exocrine acinar cells possess a substantial cytoplasmic pool of Na,K-ATPase activity [2, 20, 35], it was of interest to learn whether some fraction of this pool might be available for recruitment to the plasma membranes. Therefore, we have surveyed the Na,K-ATPase subcellular distribution in resting lacrimal gland fragments and in lacrimal gland fragments that had been stimulated with carbamylcholine for 30 min. The results indicate that the secretagogue triggers a significant redistribution of Na,K-ATPase.

Materials and Methods

Tissue Preparation

All experiments were performed with the tissue fragment preparation described by Putney et al. [28]. In each experiment, eight male Sprague-Dawley rats were killed by cervical dislocation. Lacrimal glands were removed, immediately sliced into 5-mg fragments, and placed in a modified Krebs improved Ringer's I solution (KRB) containing 5 mM Na-β-hydroxybutyrate, 0.2 mM phenylmethylsulfonylfluoride (PMSF), and 9 μg/ml Aprotinin. The KRB was kept at 37°C and continuously gassed with 95% oxygen/5% carbon dioxide. The pH of the KRB was monitored continuously; when necessary, 100% CO_2 was added to maintain pH between 7.35 and 7.45. The fragments were placed in a basket constructed of nylon mesh and a bottomless polypropylene beaker. The basket was used to transfer the fragments to fresh 40-ml aliquots of KRB at 5-min intervals throughout the in vitro incubation procedure. The in vitro incubation was performed with continuous gassing in a 37°C shaking water bath. By the end of an initial 55-min equilibration period, macromolecular secretion, marked by peroxidase release, had reached a low and stable level. Fragments were then either incubated for an additional 30 min in the same buffer or for 30 min in KRB containing 10 μM carbachol.

Cell Fractionation

At the end of the in vitro incubation period, the fragments were rapidly rinsed with ice-cold isolation buffer (5% sorbitol, 5 mM histidine-imidazole buffer, 0.5 mM NaEDTA, 0.2 mM PMSF and 9 μg/ml Aprotinin, pH 7.5). They were quickly transferred to a 30 × 150 mm tube containing 18 ml isolation buffer, then homogenized with a Tissumiser® and subjected to subcellular fractionation procedures as described [20, 21]. The initial homogenate was centrifuged at 1000 × g for 10 min. The resulting pellet was resuspended in isolation buffer, homogenized, and again centrifuged at 1000 × g for 10 min. Two additional cycles of resuspension, homogenization and centrifugation were performed. The four low-speed supernatants were pooled and collectively designated S_o. The sorbitol concentration of S_o was brought to 55% by addition of a concentrated sorbital stock solution. S_o was then loaded into a Beckman Z-60 zonal rotor and subjected to equilibrium centrifugation on a two-stage (35–55% and 55–70%) hyperbolic sorbitol density gradient.

At the end of the centrifugation, the rotor contents were displaced with 80% sucrose and collected in 14.5-ml fractions. Each fraction was diluted with 10 ml isolation buffer and centrifuged at 250,000 × g for 75 min. The resulting pellets were resuspended in isolation buffer. The supernatants were pooled into six groups, or *density windows*, as described under Results, their volumes were measured, and aliquots were taken for marker determinations. Collectively, the high-speed pellets comprised the ΣP_i *sedimentation window*, and the supernatants represented the ΣS_i *sedimentation window* [19].

Analytical Methods

Peroxidase was measured according to the procedure of Herzog and Fahimi [9]. Procedures described by Mircheff et al. [21] were used to analyze the biochemical markers of all subcellular fractions. Data are presented as percentages of the total activities recovered in the three terminal *sedimentation windows*, P_o, ΣP_i, and ΣS_i. Cumulative enrichment factors [20] at each stage of the separation may be calculated as ratios of percentage of recovered marker activity to percentage of recovered protein. Differences in the marker contents of fractions from resting and from stimulated preparations were tested for significance with the unpaired t-test.

Reagents

Sorbitol was analyzed reagent grade from J.T. Baker, and carbachol was from Sigma. All other chemicals were reagent grade and were obtained from standard suppliers.

[1] If the pump kinetic parameters remain unchanged, the Michaelis-Menten relationship derived by Lewis and Wills (1983), i.e.,

$$J = \frac{J_{max}}{1 + (K_{Na}/a_{Na})^3}$$

predicts that an increase of a_{Na} from 6 to 14 mM would increase the Na,K-ATPase pump rate from 0.7 to 0.50 J_{max}. This would substantially, but not completely, account for a 10- to 12-fold acceleration of Na^+ influx.

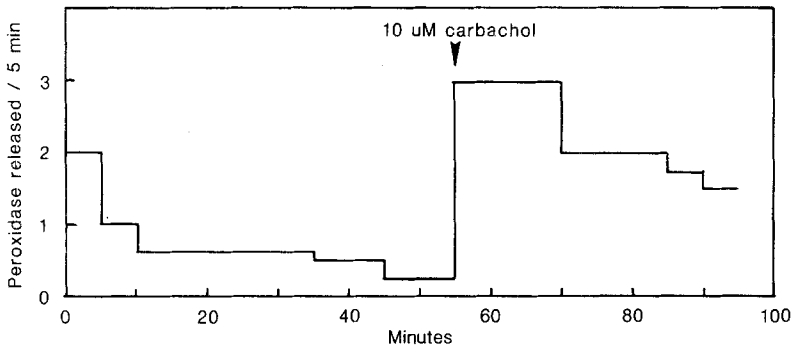

Fig. 1. Stimulation-induced release of peroxidase. As described under Materials and Methods, lacrimal gland fragments were transferred to fresh aliquots of KRB at 5-min intervals throughout the in vitro incubation period; after 55 min, the KRB contained 10 μM carbachol. Peroxidase released into the KRB is presented in arbitrary units. In four separate preparations, stimulated caused 10- to 14-fold increases in the initial rate of peroxidase release

Table 1. Distribution of markers after differential sedimentation[a]

	Total activity	P_o	ΣP_i	ΣS_i	Recovery
Na,K-ATPase					
Resting (4)	13.6 ± 1.4	2.5 ± 1.3	68.0 ± 7.2	29.5 ± 8.3	103.0 ± 27.0
Stimulated (4)	16.7 ± 2.1	1.8 ± 0.9	73.4 ± 9.1	24.8 ± 9.4	82.9 ± 7.1
Alkaline phosphatase					
Resting (4)	78.5 ± 8.6	4.0 ± 1.1	56.8 ± 5.6	39.2 ± 6.6	76.7 ± 14.5
Stimulated (3)	91.7 ± 27.2	3.3 ± 1.2	57.9 ± 1.6	38.7 ± 1.9	74.5 ± 16.6
Acid phosphatase					
Resting (4)	373.8 ± 24.9	1.9 ± 0.5	52.9 ± 1.7	45.1 ± 2.1	86.5 ± 2.4
Stimulated (3)	474.6 ± 115.0	1.7 ± 0.6	61.0 ± 8.2	37.3 ± 8.1	81.3 ± 24.2
Succinate dehydrogenase					
Resting (4)	16.0 ± 2.9	5.1 ± 1.2	91.1 ± 1.6	3.8 ± 2.3	137.9 ± 33.8
Stimulated (4)	17.5 ± 4.6	12.3 ± 11.9	84.9 ± 11.7	2.8 ± 1.1	121.6 ± 51.5
Protein					
Resting (4)	332.9 ± 36.1	1.1 ± 0.3	30.3 ± 1.7	68.6 ± 1.8	89.8 ± 4.5
Stimulated (4)	324.1 ± 33.9	1.3 ± 0.6	28.0 ± 4.9	70.8 ± 5.0	104.2 ± 9.1

[a] Total enzymatic activities in $P_o + S_o$ are expressed in units of μmol/hr; total protein is in mg. Values for P_o, ΣP_i, and ΣS_i are percentages of the total activities recovered from $S_o + P_o$. The percentage recoveries from S_o, i.e., $(\Sigma P_i + \Sigma S_i)/S_o$ are also given. Values in parentheses are number of preparations in which data were collected. All data are presented as mean ± standard deviation.

Results

Stimulation of Lacrimal Gland Fragments

The lacrimal gland fragment preparation described under Materials and Methods was developed by Putney et al. [28] for studies of stimulus-secretion coupling, and the ability of agonists to elicit release of peroxidase provides a useful indication of the integrity of the lacrimal stimulus-secretion coupling mechanism. The rate of peroxidase release as a function of time is presented in Fig. 1. Release declined to a stable baseline within 45 min of in vitro incubation; it was then accelerated 11- to 14-fold by the addition of 10 μM carbachol. This response compares well with the 10- to 15-fold increases reported by other workers [4, 28].

The morphological integrity of cells in the gland fragments was confirmed by light microscopy. The acini retained normal morphology through the in vitro equilibration and stimulation periods, although some acinar cells both in resting and in stimulated fragments exhibited randomly distributed intracellular vacuoles. Stimulation with carbachol caused a major reduction in the number of secretory granules, and, as has been noted by others [13, 18], it led to the formation of large vacuoles in the apical cytoplasm. Stimulation caused no obvious alterations in the appearance of other membranous organelles.

Differential Sedimentation

The differential sedimentation steps of the fractionation procedure define three *windows*. Distributions of markers among these *sedimentation windows* are summarized in Table 1. As in previous studies [20], ΣP_i accounted for major fractions of all recovered

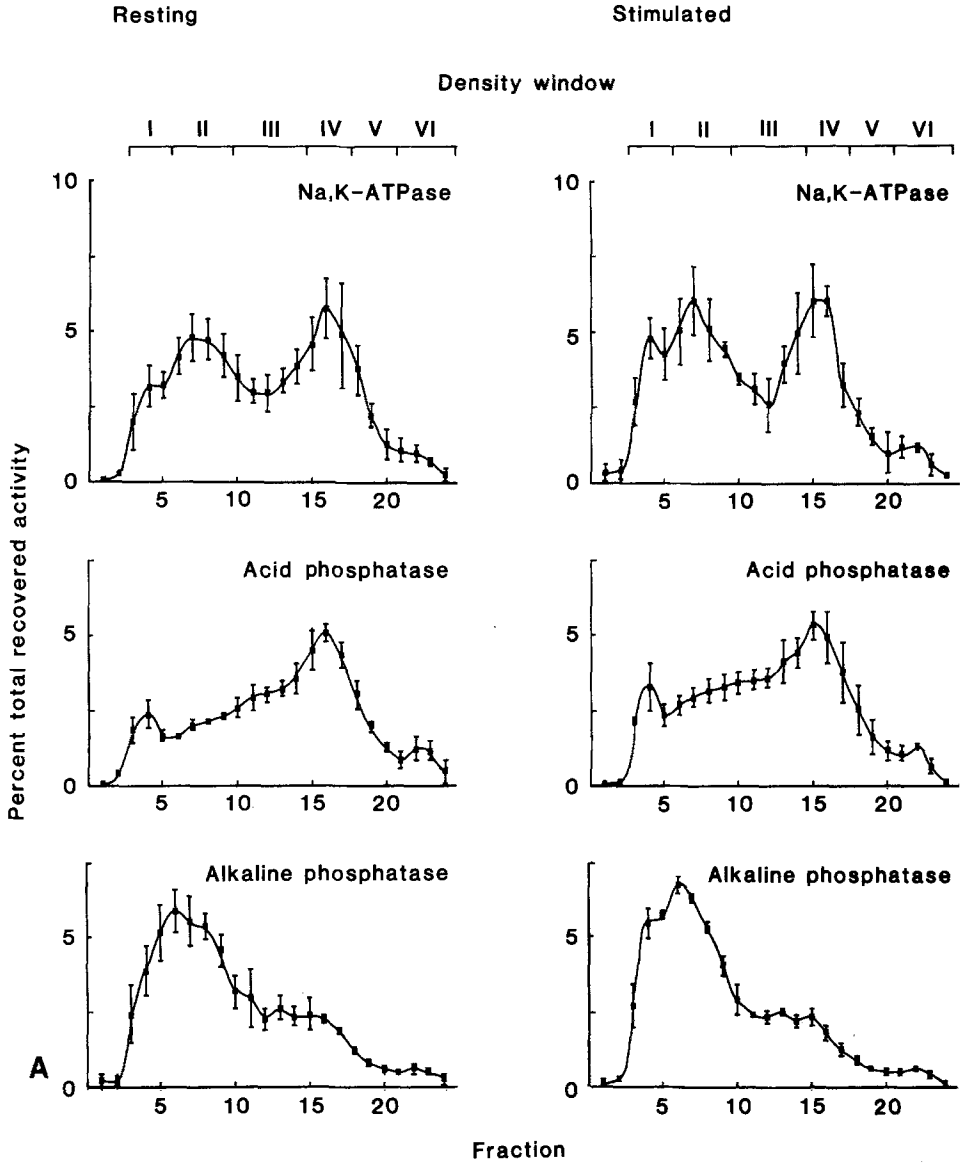

Fig. 2. Effects of stimulation with 10 μM carbachol on biochemical marker density distributions within the ΣP_i *sedimentation window*. Lacrimal gland fragments were incubated in KRB as described under Materials and Methods. After the 55-min equilibration period, they were incubated for additional 30-min periods either in the absence of carbachol (*Resting*) or in the presence of 10 μM carbachol (*Stimulated*) and then subjected to subcellular fractionation. Each 14.5-ml density gradient fraction was diluted with 10 ml isolation buffer, then centrifuged at 250,000 × *g* for 75 min. The resulting pellets comprise ΣP_i. Values presented are the means ± standard deviations from four resting preparations and four stimulated preparations. Thirty-minute stimulation led to a 1.4-fold increase in the total Na,K-ATPase activity of the fractions which comprise *density window I* and a decrease in the Na,K-ATPase activity of the fractions comprising *density window V*

enzymatic marker activities, while ΣS_i contained most (i.e. 70%) of the recovered protein. However, ΣS_i contained relatively larger, and ΣP_i smaller, percentages of the recovered activities than the fractions that were obtained with isolation media lacking protease inhibitors [20, 21]. It seems reasonable to suggest that ΣS_i contains proteases which, in the absence of inhibitors, partially inactivate enzymes that migrate in this *sedimentation window*.

Stimulation was associated with a 1.2-fold increase ($P < 0.05$) in the Na,K-ATPase activity of the initial homogenate. This observation raises the possibility that Na,K-ATPase units might be activated in some way. However, such activation must have been reversed during the subsequent fractionation steps, since Na,K-ATPase recovery from stimulated preparations was 0.8-fold the recovery from resting preparations, and the same total activi-

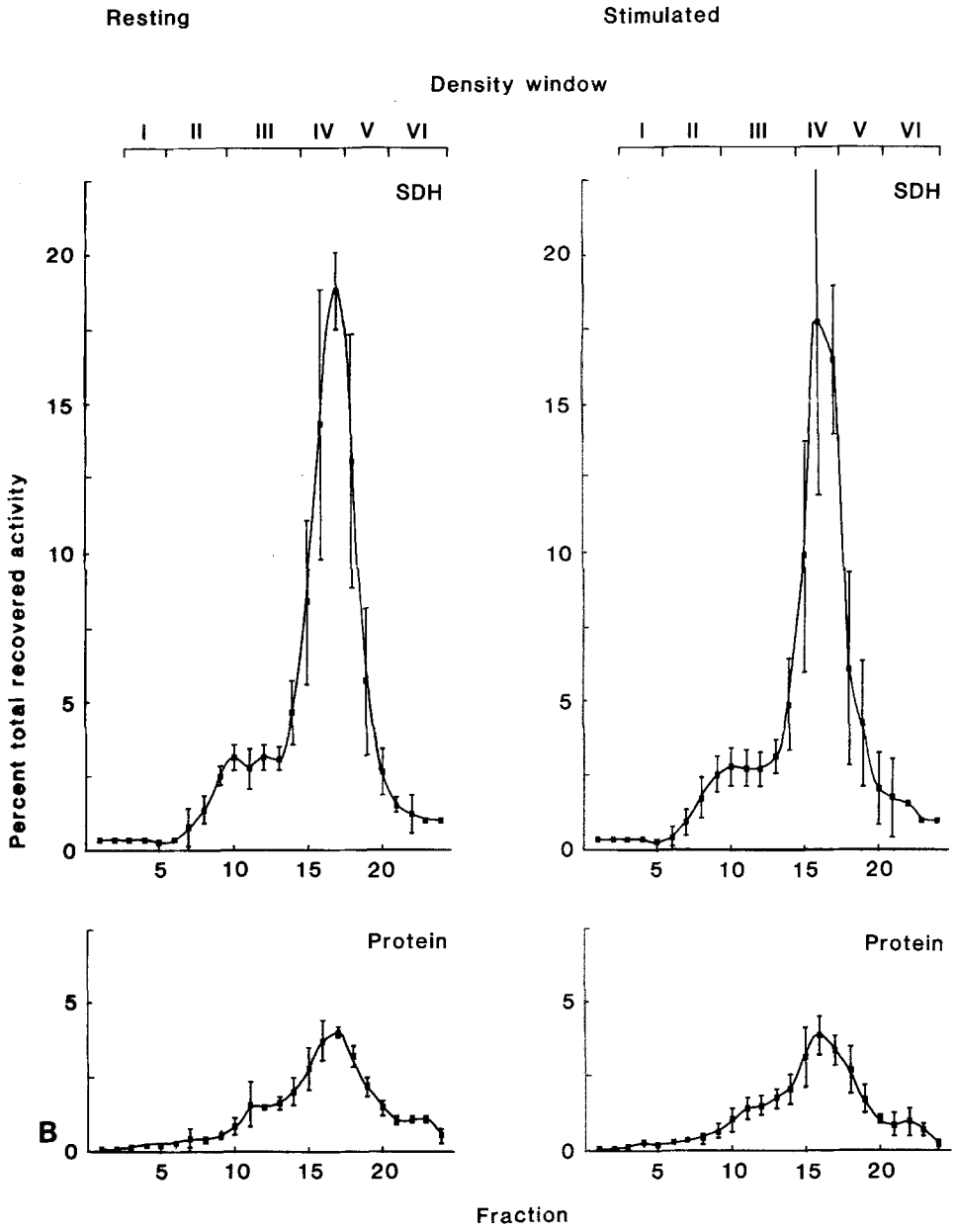

Fig. 2. *Continued.*

ties were recovered from resting (13.3 μmol/hr) and stimulated (13.4 μmol/hr) preparations. Thus, the apparent activation phenomenon was not investigated further. Stimulation was also associated with a redistribution of acid phosphatase from ΣS_i to ΣP_i, so that ΣP_i contained 52.9 ± 1.7% of the activity recovered from resting preparations and 61.0 ± 8.2% of the activity recovered from stimulated preparations ($P < 0.05$). The total activities of other markers and their distributions between ΣS_i and ΣP_i were not significantly influenced by stimulation.

Density Distributions—High-Speed Pellets

Figure 2 summarizes the distributions of biochemical markers within the ΣP_i *sedimentation window*. These distributions are similar to those obtained in earlier studies [20, 21], and, as before, six *density windows* were defined on the basis of salient features of the marker distribution patterns; the marker contents of these *density windows* are summarized in Table 2.

Thirty-minute stimulation with carbachol led to

Table 2. Distribution of markers after density gradient centrifugation[a]

	I	II	III	IV	V	VI
Na,K-ATPase						
Resting (4)	8.3 ± 1.7	17.8 ± 2.6	16.5 ± 1.6	15.2 ± 2.4	7.2 ± 1.2	3.0 ± 0.5
Stimulated (4)	11.7 ± 2.1*	20.6 ± 3.0	17.1 ± 2.6	14.1 ± 3.5	4.9 ± 0.7*	3.3 ± 1.1
Alkaline phosphatase						
Resting (4)	11.4 ± 2.6	21.2 ± 2.1	13.3 ± 0.7	6.5 ± 0.7	2.5 ± 0.2	1.8 ± 0.3
Stimulated (3)	13.8 ± 0.9	22.2 ± 0.3	12.3 ± 0.2	5.4 ± 0.7	2.0 ± 0.3	1.7 ± 0.2
Acid phosphatase						
Resting (4)	5.8 ± 0.8	8.0 ± 0.3	15.2 ± 0.5	13.8 ± 0.6	6.2 ± 0.7	3.6 ± 0.4
Stimulated (3)	7.7 ± 1.0*	12.0 ± 1.4*	19.0 ± 2.1*	13.9 ± 2.3	5.3 ± 1.7	3.1 ± 0.5
Succinate dehydrogenase						
Resting (4)	0.8 ± 1.3	4.9 ± 1.2	16.5 ± 1.1	41.6 ± 8.1	21.4 ± 7.4	4.7 ± 3.1
Stimulated (4)	0.9 ± 1.2	5.5 ± 1.7	16.1 ± 3.3	44.1 ± 10.0	12.3 ± 4.6	5.2 ± 6.5
Protein						
Resting (4)	0.5 ± 0.1	1.5 ± 0.5	7.4 ± 0.9	10.4 ± 1.4	6.8 ± 0.9	3.7 ± 0.3
Stimulated (4)	0.6 ± 0.1	1.5 ± 0.6	7.7 ± 1.8	10.2 ± 2.1	5.4 ± 1.1	2.6 ± 1.0

[a] Marker contents of the *density windows* defined in Fig. 2 are given as percentages of the total recovered activities, mean ± standard deviation. Statistically significant differences between values for resting and stimulated preparations are indicated by asterisks.

several notable changes in the marker density distribution patterns. The data presented in Table 2 indicate that stimulation increased the Na,K-ATPase content in *window I* by a factor of 1.4 ($P < 0.05$), while, as noted above, it caused no significant change in either the total amount of Na,K-ATPase recovered or the distribution of Na,K-ATPase between ΣP_i and ΣS_i. The stimulation-associated increase in the Na,K-ATPase content of *window I*, which represented 3.5% of the total recovered Na,K-ATPase activity, could be attributed to a redistribution of activity from *density windows IV* and *V*. The combined decreases in the Na,K-ATPase contents of these *windows* (3.9% of the total recovered activity) would be sufficient to account for the increase noted in *window I*; the decrease in *window V* (representing 2.3% of the total recovered activity) was highly significant ($P < 0.005$), while the apparent decrease in *window IV* was not statistically significant.

In a separate series of experiments, four lacrimal fragment preparations were fractionated immediately after the 55-min *in vitro* equilibration period, and 17 preparations were fractionated after an additional 5-min incubation with 10 μM carbachol. This brief stimulation caused a 1.3-fold ($P < 0.05$) increase in the Na,K-ATPase content of *window I*, suggesting that the redistribution of Na,K-ATPase was largely complete within 5 min of stimulation.

The increase of the Na,K-ATPase content in *window I* after 30-min stimulation was accompanied by a 1.3-fold increase ($P < 0.05$) of the *window I* acid phosphatase activity. While the Na,K-ATPase yield increased significantly only in *window I*, the acid phosphatase yield also increased in *window II* (1.5-fold) and in *window III* (1.3-fold). Furthermore, the redistribution of acid phosphatase could be accounted for almost quantitatively by the shift of activity from ΣS_i to ΣP_i noted above (Table 1). Thus, fundamentally different processes must have been involved in the redistributions of Na,K-ATPase and acid phosphatase.

The pronounced changes in the distributions of Na,K-ATPase and acid phosphatase were accompanied by a modest leftward shift in the distribution of succinate dehydrogenase and no significant change in the distributions of alkaline phosphatase and protein. Surveys of the NADPH-cytochrome *c* reductase and galactosyltransferase distributions in one resting and one stimulated preparation (*data not shown*) suggested possible stimulation-associated changes within *windows III-V*; these changes did not involve *density window I*.

Density Distributions—High-Speed Supernatants

There were no significant stimulation-associated differences in the Na,K-ATPase contents of the high-speed supernatant fractions from the various *density windows*. The acid phosphatase contents of the high-speed supernatants from *density windows I*, *II*, and *III* (Fig. 3) were not significantly changed by stimulation, indicating that there was no net change in the efficiency of sedimentation of the acid phosphatase-containing membranes which equilibrated in this region of the density gradient. Rather,

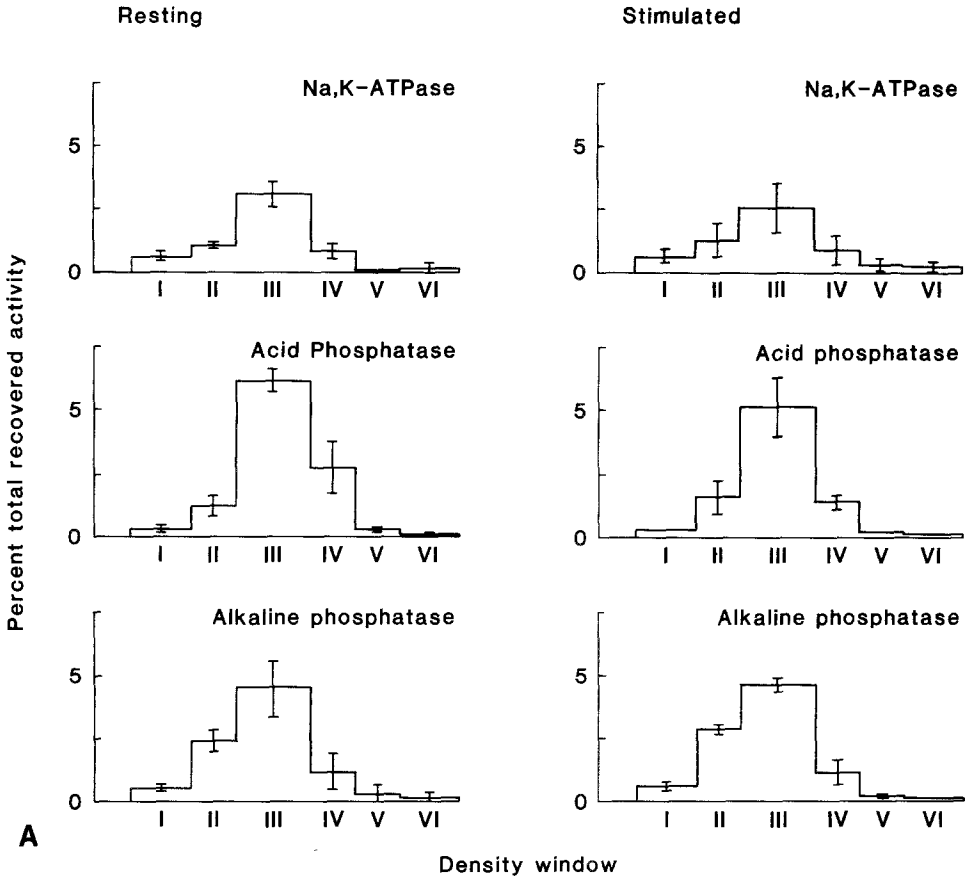

Fig. 3. Effects of stimulation with 10 μM carbachol on biochemical markers density distributions within the ΣS_i *sedimentation window*. Following high-speed centrifugation as described in the legend to Fig. 2, the supernatants were pooled into the *density windows* described. Marker contents were divided by the number of fractions per *window*, so that distributions could be presented on the same scale as in Fig. 2. Values presented are the means ± standard deviations. *Density window III* corresponded to the sample-loading layer of the density gradient, so ΣS_{III} and, to some extend, ΣS_{IV} are the loci of soluble components of the homogenate. In a separate series of experiments (Yiu, *unpublished data*), samples of ΣS_{III} were diluted further, then centrifuged at high speed. Most of the protein remained in the supernatant, while at least 80% of the Na,K-ATPase, alkaline phosphatase, and acid phosphatase activities sedimented, indicating that these activities are associated with one or more populations of membranes which have sedimentation properties distinct from those resolved in the ΣP_i *sedimentation window* (Fig. 2). Stimulation was associated with a significant decrease in the acid phosphate contents of ΣS_{IV} and ΣS_V, and these decreases were sufficient to account for the increased acid phosphatase contents of the membranes from *density windows, I, II,* and *III* (Fig. 2 and Table 2). *Fig. 3 continued on next page*

stimulation led to significant decreases in the acid phosphatase contents of the supernatants from *windows IV* and *V*. This result suggests that the acid phosphatase activity which redistributed to *windows I, II,* and *III* of the high-speed pellet fraction was derived from membranes which migrated to *IV* and *V* during density gradient centrifugation but which remained in the supernatant fractions during the subsequent high-speed differential centrifugation step.

Discussion

The stimulation-associated redistribution of Na,K-ATPase activity between *density window V* and *density window I* (Fig. 2) indicates that pump units were recruited from a cytoplasmic structure, probably a domain of the Golgi complex, and inserted into the basal-lateral plasma membrane. This interpretation depends on inferences about the subcellular origins of isolated membrane populations which follow from data presented above and from analyses in which individual *density windows* were subfractionated by partitioning in aqueous polymer two-phase systems [19, 20, 21]. It should be noted that the original density-partitioning analyses were performed with fresh parenchymal scrapings [19, 20, 21]; however, the overall picture is congruent with the results of recent analyses of resting lacrimal gland fragments (*unpublished*).

Several characteristics of the membranes equil-

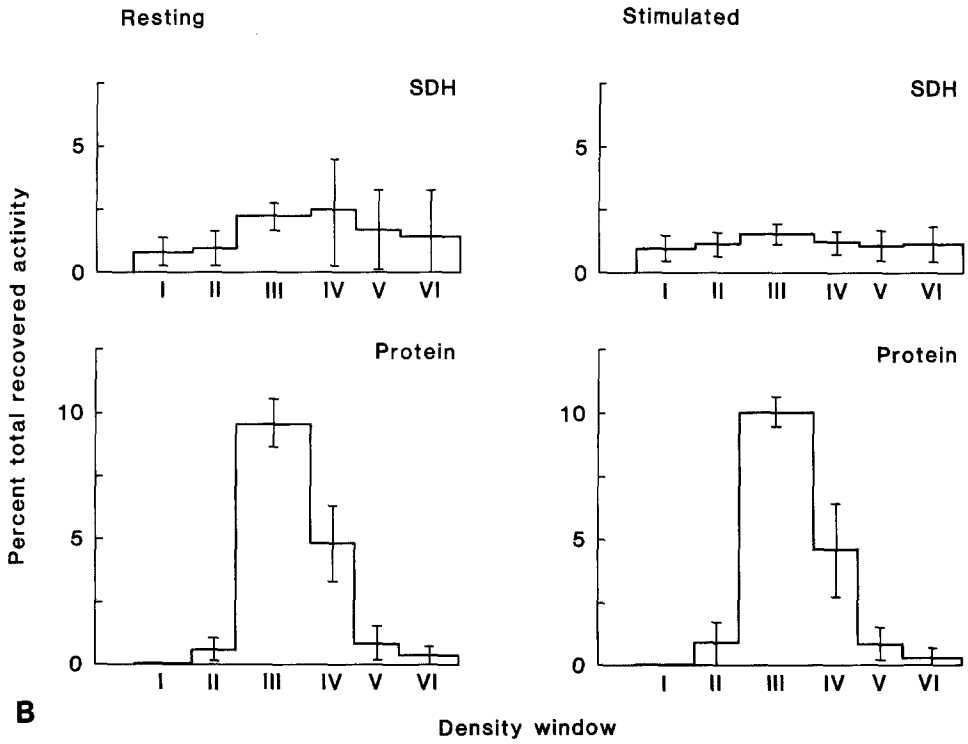

Fig. 3. *Continued.*

ibrating in *density window I* indicate that they were derived from the acinar cell basal-lateral plasma membrane. Virtually all of the Na,K-ATPase activity in *density window I* can be assigned to a single membrane population. This population is the locus of the largest observed cumulative enrichment factors for Na,K-ATPase and alkaline phosphatase (Table 2); it is also the locus of the highest observed ratio of Na,K-ATPase to galactosyltransferase [21]. Since a population with similar properties has been resolved in preliminary analyses of isolated lacrimal acini (S.C. Yiu, *unpublished*), it appears that these membranes were not derived, as had been suggested earlier [21], from a minor cell type.

Windows IV and *V* contain a multiplicity of distinct membrane populations [20]. A negligible fraction of the Na,K-ATPase activity can be assigned to the mitochondria, marked by succinate dehydrogenase (Fig. 2), which can be resolved by differential rate centrifugation [20]. A small fraction of the total Na,K-ATPase activity, at a cumulative enrichment factor of 0.5, can be assigned to the major population of endoplasmic reticulum-derived membranes, marked by NADPH-cytochrome *c* reductase, which spans *windows III-V* (*unpublished*). A similarly small fraction can be assigned to a group of microsomal membrane populations which is characterized by high partition coefficients in dextran-polyethyleneglycol two-phase systems and by high contents of alkaline phosphatase and acid phosphatase relative to the other enzymatic markers [20]. Most of the Na,K-ATPase activity is associated with an additional group of microsomal populations which is characterized by intermediate partition coefficients and by high contents of galactosyltransferase relative to the other markers [20]. Their high specific contents of galactosyltransferase suggest that these populations were derived from the Golgi complex, while differences in their density and partitioning properties and in their relative contents of the other markers suggest that they originated from biochemically distinct domains. Because this group of populations accounts for most of the Na,K-ATPase in *windows IV* and *V*, it is likely that one or more of them represents the domain from which Na,K-ATPase is recruited in response to stimulation. In this respect, the cytoplasmic locus of Na,K-ATPase available for recruitment to the plasma membranes is analogous to that of the insulin-dependent glucose transporter [12]; it appears not to be a population of preformed vesicles such as are thought to exist for the proton-translocating ATPases [8] and ADH-sensitive water channels [17].

Estimates of the precise role which Na,K-ATPase recruitment plays in acinar Na^+ homeosta-

sis hinge on the question of whether the Na,K-ATPase-rich population equilibrating in *window I* represents the entire acinar cell basal-lateral membrane. The uncertainty exists because the identity of the membranes equilibrating in *density window II* is ambiguous. As in *window I*, most of the Na,K-ATPase activity equilibrating in *window II* can be attributed to a single membrane population [20]. However, this population exhibits characteristics expected both of plasma membranes and of Golgi membranes. That is, it has relatively large cumulative enrichment factors for Na,K-ATPase and alkaline phosphatase (Table 2), but it also has a relatively large cumulative enrichment factor for galactosyltransferase; compared to the Na,K-ATPase-rich population from *window I*, it has three- to fourfold higher ratio of galactosyltransferase to Na,K-ATPase [20]. Although we previously concluded that this population was derived from acinar cell basal-lateral membranes [21], we must now entertain the possibility that it was derived from a domain of the Golgi complex involved in the assembly or recycling of basal-lateral membrane constituents. Such a suggestion is consistent with recent information on the recycling of plasma membrane glcopeptides [6]; the magnitude of the cytoplasmic pool of Na,K-ATPase implicit in this suggestion is, at least qualitatively, consistent with the size of cytoplasmic Na,K-ATPase pools measured by immunochemical techniques [34]. In short, if the Na,K-ATPase-containing populations of *windows I* and *II* represent different microdomains of the acinar cell basal-lateral membrane, the stimulation-associated changes summarized in Table 2 would contribute to a 1.2-fold increase in the plasma membrane Na,K-ATPase J_{max}. If, as now seems more likely, only the population in *window I* represents the basal-lateral membrane, recruitment from cytoplasmic membranes would increase the plasma membrane J_{max} 1.4-fold.

There are many examples of transport activities which are redistributed between cytoplasmic sites and cell surface membranes during rapid changes in cellular function. These include proton-translocating ATPases [7, 8], ADH-sensitive water channels [17], insulin-dependent glucose transporters [12], Na^+ channels [15], and system A amino-acid transporters [5]. There also have been several previous suggestions that exocytotic insertion and endocytotic retrieval mechanisms might regulate plasma membrane Na,K-ATPase pump activities. For example, rapid insertion of additional pump units could account for the fact that marked increases in transepithelial Na^+ fluxes occur in small intestine [10] and gallbladder [22] without substantial or sustained perturbations of steady-state cytosolic a_{Na}. Exocytotic insertion has also been implicated in the insulin-dependent stimulation of Na^+ efflux from skeletal muscle [11]. Conversely, endocytotic retrieval of pumps might account for the observation that decreased Na^+ entry into toad bladder epithelial cells led to decreased transepithelial flux with no change in steady-state cytosolic a_{Na} [14]. Thus, the translocation of Na,K-ATPase between cytoplasmic sites and the plasma membranes is consistent with observations from a number of different cell types.

The recruitment of Na,K-ATPase is accompanied by a redistribution of acid phosphatase, such that the acid phosphatase activity of membranes equilibrating in *density windows I*, *II*, and *III* increases. It is, of course, possible to imagine scenarios in which the two redistribution phenomena are functionally related, but it should also be recalled that a number of different membrane translocation events are known to be triggered by secretagogues. These include exocytotic release of macromolecular secretory products and subsequent endocytotic retrieval of secretory vesicle membranes, as well as endocytotic internalization from the acinar cell basal-lateral membrane [24, 25]. The internal redistribution of acid phosphatase could well be a manifestation of one or more of these processes; in any event, it serves to highlight the complexity of the membrane traffic which accelerates when acinar cells are stimulated to secrete electrolytes and macromolecular products.

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