Synergistic Receptor-Activated Calcium Increases in Single Nonpigmented Epithelial Cells

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PURPOSE. To determine whether single nonpigmented ciliary body cells contain the signaling mechanism to produce synergistic drug-activated increases in Ca\(^{2+}\), or whether these responses are produced cooperatively by interaction among groups of cells.

METHODS. Suspensions of single nonpigmented cells were plated onto soft collagen gels. Fura-2 fluorescence ratio imaging was used to examine receptor-evoked changes in intracellular Ca\(^{2+}\) concentration.

RESULTS. Nonpigmented cells plated on soft collagen gels retained a rounded shape with membrane evaginations visible on their surface. Application of acetylcholine (10 \(\mu\)M) or epinephrine (1 \(\mu\)M) each produced small increases in intracellular Ca\(^{2+}\), but in combination they produced a Ca\(^{2+}\) increase of more than 10-fold. This synergistic Ca\(^{2+}\) increase was a result of activation of muscarinic and \(\alpha_2\)-adrenergic receptors because a specific \(\alpha_2\)-adrenergic agonist could substitute for epinephrine in producing the response. The response could be blocked by a specific \(\alpha_2\)-antagonist and a muscarinic antagonist. An \(\alpha_2\)-agonist could not substitute for epinephrine in producing a synergistic increase, nor could the synergism be blocked by \(\alpha_4\) or \(\beta\)-antagonists. The Ca\(^{2+}\) increase was largely produced by release from internal stores, because the peak amplitude of the response was nearly the same in the external solution containing a low Ca\(^{2+}\) concentration; however, the influx of Ca\(^{2+}\) into the cell was responsible for maintenance of a steady component of the Ca\(^{2+}\) increase during maintained drug stimulation and for refilling the internal stores.

CONCLUSIONS. Single nonpigmented cells can produce synergistic increases in Ca\(^{2+}\) on multiple receptor activation, indicating that the mechanism of synergism does not require the interaction of multiple cells. The Ca\(^{2+}\) increase is a result of release from internal stores and Ca\(^{2+}\) entry through an as yet undefined conductance or transport system in the plasma membrane. (Invest Ophthalmol Vis Sci. 1998;39:1429–1435)

Changes in the intracellular free Ca\(^{2+}\) concentration are widely recognized as regulators of cellular function.1 These changes have been shown to play an important role in the pharmacologic responses of ocular epithelia, including ciliary body.2-3 A variety of pharmacologic agents have been shown to produce an increase in intracellular Ca\(^{2+}\) concentration in ciliary body epithelium.4-9 In addition, certain receptors when stimulated in combination can produce large synergistic increases in Ca\(^{2+}\).7-11 These synergistic Ca\(^{2+}\) increases can be recorded from intact processes7,10-11 and even from the isolated nonpigmented (NP) cell layer of this bilayered epithelium.8,9 The mechanism and function of these synergistic increases remain unclear.

To understand the generation of Ca\(^{2+}\) increases in ciliary body, it is useful to study responses from single, isolated cells. However, when single cells are isolated from one another and grown in culture on rigid supports, the synergistic Ca\(^{2+}\) increase is lost, even though the receptors for the transmitters continue to be present and to produce increases in Ca\(^{2+}\). Yet, cultured cells can regain the synergism if they are grown so that they have the cell shape and cytoarchitecture of the cells in vivo. We have previously shown that this occurs when NP cells are grown as monolayers on a deformable substrate, such as a collagen raft.9 However, it remains unclear whether the synergistic responses are produced by single cells functioning independently or by groups of cells working in concert, because the cells of the ciliary body epithelium are connected by numerous gap junctions.12 These junctions have been shown to pass the signal produced by the simultaneous activation of acetylcholine and epinephrine receptors from one layer to another.13

To investigate whether multiple cellular interaction is required to produce the synergistic response, we have developed a technique for plating single cells onto soft collagen gels. These gels are too easily deformed to support the traction necessary for cell spreading.14-16 Single cells under these conditions maintain a rounded shape, and we show that they also retain a synergistic response to simultaneous muscarinic and \(\alpha\)-adrenergic activation. This is the first demonstration that single NP cells can be grown so as to maintain synergistic Ca\(^{2+}\) increases, and we have used this preparation to examine the role of intracellular stores and Ca\(^{2+}\) influx in producing the pharmacologic responses of these cells.

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METHODS

Cell Culture

Pure populations of NP ciliary body epithelial cells were prepared as previously described with slight modification in the enzyme and substrate used, as follows. Nonpigmented cell layers were isolated from pigmented rabbits aged 7 days to 4 weeks. All procedures used were in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The NP cell layers were enzymatically digested into a single cell suspension with 0.2% collagenase—dispase (Boehringer Mannheim, Ingelheim, Germany) or 20 U/ml papain (Worthington Biomedical, Freehold, NJ) for 20 to 30 minutes at 37°C. The cells were washed and subsequently plated onto collagen gels at low density so that they would remain isolated from neighboring cells.

The collagen gels were made from a solution of collagen type I (Collaborative Biomedical Products, Bedford, MA), cast according to the manufacturer’s protocol. The collagen solution was diluted to a sufficiently low concentration (usually 2%-3%) so that the gel would be soft enough to prevent the majority of cells from spreading and still provide a gel that would hold together during the recording procedures used in these experiments. The concentration had to be empirically tested for each lot of collagen solution because of the variability in the collagen content of the stock solution. The cultures were placed in an incubator at 37°C with 5% CO₂ in air until use.

Explant cultures used in a few experiments were prepared by placing pieces of isolated NP cell layers comprising approximately one fourth of a ciliary body into a culture dish, modified by gluing a glass coverslip over a 13-mm hole drilled into the bottom. The glass coverslip was coated with mouse laminin (Collaborative Biomedical Products) according to the manufacturer’s protocol.

Fura-2 Loading and Ca²⁺ Imaging

To measure changes in intracellular free calcium concentration ([Ca²⁺]ᵢ), cells were loaded with 5 μM fura-2 acetoxymethyl ester (fura-2 AM; Molecular Probes, Eugene, OR) in culture medium for 60 to 90 minutes and washed with HEPES-buffered Ringer’s solution (see below for formulation). After loading, the culture plate was placed on a heated microscope stage and superfused with Ringer’s solution or a test solution. In some experiments, particularly those in which we examined the effects of rapid changes to low-Ca²⁺ solutions, the drugs were applied through a microperfusion system consisting of either a large-bore pipette placed close to the cell or through a U-tube. Ca²⁺ was measured with an imaging system (Ion-Optix, Milton, MA). Calibration of the fluorescent signal was made after perfusion of the cells with 5 μM ionomycin in normal Ringer’s solution and in Ringer’s solution containing 10 mM EGTA. Each cell was calibrated in situ using its respective maximum and minimum fluorescent signals according to the methods of Grynkiewicz et al.

In each experiment, Ca²⁺ could be measured from multiple cells in the same visual field. There was some variability between cells in the absolute values of Ca²⁺ measured. Some of these differences may have been caused by the handling of the cultures or the accuracy of the calibration procedure. Therefore, to compare values measured among the cells, data have been given both as absolute concentrations (in nanomolar) and as percentage increases above the resting level, measured just before application of the test solution. The overshoot in [Ca²⁺]ᵢ on return to the Ca²⁺-containing solution from the low-Ca²⁺ solution is defined as the peak increase above the resting level minus the sustained increase above the resting level, divided by the sustained increase above the resting level. The resting level for this calculation is the [Ca²⁺]ᵢ in the low-Ca²⁺ solution.

Solutions

HEPES-buffered Ringer’s solution (pH 7.6, 293–298 mOsm) was of the following composition (in millimolar): 137 NaCl, 4.3 KCl, 1.7 CaCl₂, 0.8 MgCl₂, 5 sucrose, 7 glucose, 10 HEPES, and 7 NaOH. Low-Ca²⁺ Ringer’s solution was prepared by substituting 10 mM EGTA for equimolar NaCl (calculated free extracellular Ca²⁺ concentration, <3 nM). Acetylcholine, methoxamine, yohimbine, and 5-bromo-N-(4,5-dihydro-1H-imidazol-2-yl)-6-quinoxalinamine (UK14,304) were purchased from Research Biochemicals (Natick, MA). All other chemicals were from Sigma Chemical (St. Louis, MO). All drugs were prepared as concentrated stocks and stored at −20°C.

RESULTS

Drug Responses in Isolated Cells

Individual NP cells, which were round with membrane evaginations on their surface, were used to examine changes in [Ca²⁺]ᵢ in response to the application of 10 μM acetylcholine (ACh) and/or 1 μM epinephrine (Epi). The cells were used from 2 to 3 hours to 2 weeks after plating. Because we had difficulty loading cells with fura-2 within 1 day of plating, we made the majority of our measurements from cells that had been in culture for 2 to 14 days. All the cells included in these measurements were sufficiently far away from their nearest neighbors so that direct communication between cells was unlikely to have contributed to the results.

The resting level of free Ca²⁺ in individual NP cells plated on collagen gels was 55 ± 5 nM (mean ± SEM, n = 110). Exposure to 10 μM ACh or 1 μM Epi caused a small increase in Ca²⁺ (Fig. 1). ACh increased the [Ca²⁺]ᵢ to a mean level of 212 ± 16 nM (313% ± 31% over the baseline, n = 95). The response to ACh lasted as long as the drug was present. In most cases, a small transient peak preceded this sustained Ca²⁺ increase. Epi had a similar, though somewhat smaller effect, increasing the [Ca²⁺]ᵢ to a mean level of 194 ± 24 nM (250% ± 37%, n = 66). The response to Epi was more transient, falling to the resting level even in the presence of the drug.

We have previously reported a synergistic increase in [Ca²⁺]ᵢ when muscarinic and adrenergic receptors are activated in intact ciliary processes, isolated NP cell layers, and monolayer cultures of NP cells grown on collagen gels. This synergistic mechanism is also observed in single NP cells (Fig. 1). In 71% of the isolated cells grown on collagen gels from which measurements were made, the combined application of ACh and Epi produced a synergistic increase in the free Ca²⁺ to 1610 ± 147 nM (n = 86), or 2902% ± 224% over the baseline concentration. The response consisted of a large transient peak that rapidly fell to a small sustained level that lasted as long as the drugs were present. For this group of cells, the free Ca²⁺ during the sustained portion of the response was 268 ± 26 nM, approximately 524% ± 61% of the baseline level.
Synergistic Effect in Single Nonpigmented Cells

Intracellular Ca\(^{2+}\) increase in response to the application of 10 \(\mu\)M acetylcholine (bar ACh), 1 \(\mu\)M epinephrine (bar Epi), or ACh and Epi combined (bar ACh/Epi) in a single nonpigmented ciliary body epithelial cell loaded with fura-2. The response to ACh or Epi was small, whereas the combined drugs elicited a massive increase composed of a transient peak and a small sustained level. The inset shows the response to ACh or Epi on an expanded scale.

In cells not showing a synergistic response, the peak response to the combined drugs was 359 ± 41 nM (640% ± 82% over the baseline, \(n = 35\)), which was not significantly different from a purely additive response.

The Response to Acetylcholine and Epinephrine Is Mediated by Muscarinic Cholinergic and \(\alpha_2\) Adrenergic Receptors

We have previously shown in intact tissue that the pharmacologic subclass of adrenergic receptor responsible for the synergistic response is of the \(\alpha_2\) subtype. Figure 2 shows a similar characterization for the response in single cells. Application of a 1-\(\mu\)M concentration of the specific \(\alpha_2\)-agonist UK14,304 elicited a small increase of 72 ± 10 nM (59% ± 10%, \(n = 12\)). In combination with ACh, however, the response to UK14,304 was large, increasing the free Ca\(^{2+}\) concentration to 1738 ± 414 nM (2547% ± 487%, \(n = 7\)). In the cell of Figure 2A, the combination of ACh and Epi then produced an even larger increase in the [Ca\(^{2+}\)]\(_i\), although there was no significant difference, on average, between the magnitude of the response to ACh and UK, and to ACh and Epi.

The predominant \(\alpha_2\)-adrenergic nature of the response was further confirmed with the use of the \(\alpha_1\)-agonist methoxamine (Fig. 2B). Methoxamine alone increased the [Ca\(^{2+}\)]\(_i\), to 61 ± 6 nM (17% ± 9%, \(n = 5\)), and in combination with ACh the [Ca\(^{2+}\)]\(_i\) rose only to 210 ± 55 nM (534% ± 148%, \(n = 4\)), which was not significantly different from simple summation of the responses to each drug alone. Even at 75 \(\mu\)M, methoxamine failed to produce a synergistic response, although with ACh it increased the cell Ca\(^{2+}\) to 457 ± 51 nM (567% ± 35%, \(n = 5\), data not shown). These same cells did show a synergistic increase to ACh and Epi (see Fig. 2B). These results are of some interest, because they indicate that NP cells have \(\alpha_1\) receptors, which can produce an increase in intracellular Ca\(^{2+}\), even though these receptors do not interact with the pathway linked to muscarinic receptors, which produces synergistic Ca\(^{2+}\) increases. Because neither we nor Schütte et al. observed any significant change in Ca\(^{2+}\) to the \(\beta\)-agonist isoproterenol in intact tissue or NP cell explants, we did not test this drug on isolated cells.

In addition to specific agonists, muscarinic cholinergic and adrenergic antagonists were used to classify the receptors involved in the synergistic response. These experiments are shown in Figure 3. Application of atropine, a muscarinic antagonist (1 \(\mu\)M), caused a reversible block (78% ± 3%, \(n = 6\)) of the synergistic Ca\(^{2+}\) increase (Fig. 3A). In another group of cells, the \(\alpha_2\)-adrenergic antagonist yohimbine (10 \(\mu\)M) caused a reversible block of 54% ± 10% in 8 of 11 cells tested (Fig. 3B). This block was not a result of the reported action of yohimbine on Ca\(^{2+}\) entry, because yohimbine had no effect on the Ca\(^{2+}\) increase...
FIGURE 3. The synergistic Ca$^{2+}$ response to acetylcholine (ACh) and epinephrine (Epi) could be blocked with either muscarinic or $\alpha_2$-adrenergic antagonists. (A) Atropine (1 µM, bar Atr), a muscarinic cholinergic antagonist, caused a near-complete inhibition of the rise in intracellular Ca$^{2+}$ in response to ACh and Epi (bar ACh/Epi). (B) The synergistic increase in intracellular Ca$^{2+}$ was also reversibly blocked by a 10-µM concentration of the $\alpha_2$-adrenergic antagonist yohimbine (bar Yoh).

caused by the application of ACh alone (not shown). In three other cells, yohimbine failed to block the response to the application of ACh and Epi. The reason for this is unclear but may result from the presence of multiple $\alpha_2$-receptor subtypes found in ciliary epithelium, which may have different sensitivities to yohimbine. However, in seven and three cells, respectively, the $\alpha_2$-adrenergic antagonist prazosin (2.5 µM) and the $\beta$-antagonist propranolol (10 µM) were without effect. Taken collectively, these results indicate that the adrenergic receptor involved in the synergistic response is of the $\alpha_2$-subtype.

The Synergistic Response Is a Result of Calcium Release from Internal Stores and Calcium Entry

To investigate the relative contributions to the Ca$^{2+}$ increase of release from internal stores and influx across the plasma membrane, we examined the synergistic Ca$^{2+}$ response after removal of most of the extracellular Ca$^{2+}$. We achieved this by using two methods as follows.

In the first method, we exposed the cell to a bathing solution containing low-Ca$^{2+}$ (see Methods) and then applied the drugs in this same solution. The response to ACh and Epi in this case consisted solely of a transient peak, which fell rapidly back to the baseline even in the maintained presence of the drugs (Fig. 4A). This is unlike the responses produced in this same cell in normal Ca$^{2+}$ Ringer’s, which showed a sustained component of Ca$^{2+}$ increase for as long as the drugs were present.

For all the cells from which synergistic responses to ACh and Epi were measured after first perfusing with low-Ca$^{2+}$ medium, the intracellular free-Ca$^{2+}$ concentration at the peak of the response was 770 ± 286 nM, an increase of 1071% ± 356% (n = 9) over the level before the application of the drugs. This is, on average, 53% of the response in the presence of the normal external Ca$^{2+}$ concentration in these same cells. After the peak, the [Ca$^{2+}$], rapidly fell to 41 ± 5 nM (55% ± 6% of the baseline level), though still in the presence of ACh and Epi. This is much lower than the Ca$^{2+}$ level in these same cells during the maintained component of the response in the presence of external Ca$^{2+}$. On return to Ca$^{2+}$-containing solution, the [Ca$^{2+}$], returned to near the resting level after a brief overshoot of 26% ± 10% (see Figs. 4A, 5).

Our second method is as follows: Because it was possible that, by exposing the cells to low-Ca$^{2+}$ medium before testing the drug response, we were decreasing the intracellular pool available for release, we took advantage of our isolated cell preparation to examine the role of extracellular Ca$^{2+}$ using a fast-perfusion device. In these experiments, we applied the drugs and the low-Ca$^{2+}$ medium together with a micropipette with a tip positioned close to the cell. This produced a much more rapid solution change. When ACh and Epi were applied in this way in low-Ca$^{2+}$, the free Ca$^{2+}$ level inside the cell rose to 1333 ± 363 nM (2948% ± 780% over the previous level, n = 6) and then rapidly fell to 50 ± 9 nM (4% ± 3% over the previous level). Thus, when Epi and ACh were applied in low-Ca$^{2+}$ by rapid perfusion, the percentile increase at the peak of the response was on average equal to the response in the presence of external Ca$^{2+}$. The response without external Ca$^{2+}$ was however much faster, decaying back to baseline even in the presence of the drugs (see Fig. 4A).

These results indicate that the peak of the Ca$^{2+}$ increase produced by drug application is largely independent of the entry of extracellular Ca$^{2+}$ into the cell and reflects mostly release of Ca$^{2+}$ from internal stores. The sustained component of the response, on the other hand, seems to be primarily the result of Ca$^{2+}$ influx across the plasma membrane. To examine this question more critically, we used a fast perfusion device (a U-tube or micropipette) to apply a low-Ca$^{2+}$ solution during the sustained component, thus rapidly washing away the free Ca$^{2+}$ from the immediate vicinity of the cell (Fig. 4B). This procedure produced a rapid decrease in the intracellular Ca$^{2+}$ concentration during the sustained component of the response of 55% ± 7% (n = 11), from an average level of 337 ± 80 nM to 105 ± 13 nM. When the flow from the pipette was turned off, the [Ca$^{2+}$], rapidly returned to the level before the low-Ca$^{2+}$ perfusion.

Extracellular Ca$^{2+}$ Is Required to Refill the Stores

The results in Figure 4B indicate that influx of Ca$^{2+}$ from the extracellular solution is required to produce the major part of...
The sustained component of Ca\textsuperscript{2+} increase. This influx appears also to be required for refilling intracellular stores with Ca\textsuperscript{2+}, because these stores can be depleted in low-Ca\textsuperscript{2+} solution. The results in Figure 5 show that repeated applications of ACh in low-Ca\textsuperscript{2+} medium caused a gradual decline in the Ca\textsuperscript{2+} response. Application of a 1-μM concentration of the Ca\textsuperscript{2+} ionophore ionomycin under these conditions could still produce an appreciable rise in free Ca\textsuperscript{2+} concentration, which is unlikely to have been produced by influx across the plasma membrane because the extracellular Ca\textsuperscript{2+} concentration in this experiment was so low (<3 nM) and the tissue had been in this low-Ca\textsuperscript{2+} solution for several minutes. It seems possible that this Ca\textsuperscript{2+} came from stores that did not participate in the ACh-mediated Ca\textsuperscript{2+} release and were not depleted even by repeated application of ACh. The loss of the response to ACh in the absence of extracellular Ca\textsuperscript{2+} does not appear to be a result of desensitization of the receptor, because the response to ACh returned when Ca\textsuperscript{2+} was restored to the medium. Furthermore, the response to ACh in normal Ca\textsuperscript{2+} medium was not diminished by repeated application at intervals similar to those used in the low-Ca\textsuperscript{2+} solution.

**DISCUSSION**

In this study, we have shown that synergistic responses to muscarinic and adrenergic agonists can be elicited from single NP cells from the ciliary body. This observation demonstrates that all the components of the signal cascade necessary to produce this aspect of NP cell physiology are present in a single NP cell. This conclusion is subject to two important qualifications. First, synergistic responses are rarely seen in cells grown on rigid supports such as glass or tissue culture plastic but occur with much greater frequency in rounded cells grown (as we have shown) on soft collagen gels. We believe these rounded cells have not replicated and thus they have retained at least some of their differentiated properties, although we have not examined the amount of replication specifically. Second, even on collagen gels, only 70% of the cells have synergistic responses. It is possible that this variation represents real differences between NP cells from different parts of the ciliary body, because we have dissociated the entire NP layer for our experiments. Anatomic and possible functional differences between the different regions of the ciliary processes have been previously described by other investigators.\textsuperscript{23-26} It is also possible that, even in our best preparations, some of the cells have lost the response as an artifact of dissociation and cell culture.

The increases in Ca\textsuperscript{2+} produced by ACh and Epi resemble in many respects the responses we and others have previously recorded from NP cell explants and from isolated, intact tissue.\textsuperscript{7,8,10,11} The adrenergic component of the synergistic response could be mimicked by application of UK14,304 but not by methoxamine. In addition, the synergistic response was blocked by yohimbine but was unaffected by prazosin or propranolol. Therefore, it is likely to be mediated primarily by activation of an α\textsubscript{2} receptor instead of an α\textsubscript{1} or β receptor.

The similarity of the response in single cells to those of more intact preparations would seem to indicate that our method of dissociating and maintaining single cells on soft collagen gels is largely successful in maintaining many features of the normal pharmacology of these cells. There are, however, some differences in the behavior of single cells in our preparations and tissue in more intact preparations. One difference is that the application of UK14,304 by itself produces a small decrease in Ca\textsuperscript{2+} in intact, isolated epithelium\textsuperscript{7} for reasons that are still unclear. In single cells, however, UK14,304 produces a clear increase (see Fig. 3A), averaging 72 ± 10 nM (or 59%
Application of ACh in the presence of external Ca\(^{2+}\) caused an ACh application in low-Ca\(^{2+}\) solution gradually diminished. We pigmented cells in regulating the [Ca\(^{2+}\)] in ciliary body. Result of Ca\(^{2+}\) entry. In addition, Ca\(^{2+}\) influx appears to be served in the isolated NP layer. Also, the response to Epi when repeated applications of ACh in the low-Ca\(^{2+}\) external solution gradually decreased until it was barely detectable. This decline does not appear to be desensitization of the receptor, because repeated applications of ACh in the presence of Ca\(^{2+}\) showed no such decline. Application of ionomycin (1 \(\mu\)M) could elicit a rise in [Ca\(^{2+}\)], even after ACh could not, indicating the existence of an additional pool not mobilized by ACh. Notice the transient increase on exposure of the cell to low-Ca\(^{2+}\) Ringer’s solution and again on return to Ca\(^{2+}\)-containing solution. Each of the ACh applications was for only 30 seconds.

above the resting level). A similar increase has also been observed in the isolated NP layer. Also, the response to Epi when applied alone is much larger in single cells and explants than in intact processes. It is possible that at least some of these differences arise from the presence of the pigmented cell layer in intact tissue, if one considers that Schütte et al.\(^\text{13}\) have shown that Ca\(^{2+}\) can pass between NP and pigmented cells, presumably through the junctions that interconnect them. Thus, it is possible that the pigmented cells are acting as a Ca\(^{2+}\) sink for the Ca\(^{2+}\) increase produced by NP cells in intact tissue but not, of course, for the Ca\(^{2+}\) released in single cells. Additional experiments are required to determine the role of the pigmented cells in regulating the [Ca\(^{2+}\)], in ciliary body.

Activation of many Ca\(^{2+}\) mobilizing receptors results in a transient increase in intracellular Ca\(^{2+}\) followed by a sustained plateau phase (see Ref. 27 for review). This peak response is typically attributed to release from intracellular stores, whereas the sustained component is attributed to Ca\(^{2+}\) influx. Our experiments in Ca\(^{2+}\)-free solutions seem to confirm these observations from other cell types, because a transient peak was observed in response to ACh and Epi application even in the absence of extracellular Ca\(^{2+}\). The sustained component of the response was abolished when Ca\(^{2+}\) was removed from the bathing solution. Thus this portion of the response is likely a result of Ca\(^{2+}\) entry. In addition, Ca\(^{2+}\) influx appears to be required to refill the stores because the response to multiple ACh application in low-Ca\(^{2+}\) solution gradually diminished. We have made no attempt at this time to identify the Ca\(^{2+}\) entry pathway in these cells.

In other cell types, depletion of intracellular stores even in the absence of receptor activation results in influx of Ca\(^{2+}\) across the membrane.\(^\text{28-30}\) In single NP cells, lowering of extracellular Ca\(^{2+}\) without receptor activation resulted in either a decline in the [Ca\(^{2+}\)], or a small transient increase in intracellular Ca\(^{2+}\) (see, e.g., Figs. 4A, 5B). On return to normal external Ca\(^{2+}\), the [Ca\(^{2+}\)], overshot the resting level before low-Ca\(^{2+}\) exposure. This result indicates that a Ca\(^{2+}\) entry pathway was opened as a result of low-Ca\(^{2+}\) exposure. These changes in [Ca\(^{2+}\)], in response to low-Ca\(^{2+}\) solutions are slower and smaller than those we have observed in intact tissue. The reason for this is presently unclear but may again be attributed to the presence of the pigmented cells in intact tissues.

It is possible that agonist-induced changes in [Ca\(^{2+}\)], may have a role in regulating aqueous secretion—there is evidence that the opening of Ca\(^{2+}\)-dependent channels in many epithelia is a signal that regulates secretory activity.\(^\text{31}\) Ca\(^{2+}\)-dependent K\(^+\) channels have been shown to be present in ciliary body epithelial cells,\(^\text{32}\) and transcripts for protein kinase C-dependent Cl\(^-\) channels,\(^\text{33}\) and transcripts for protein kinase C-dependent Cl\(^-\) channels,\(^\text{34}\) which activity has also been shown to be regulated by Ca\(^{2+}\)-calmodulin-activated kinase.\(^\text{35}\) Although a direct action of Ca\(^{2+}\) on NP cell Cl\(^-\) channels has not been demonstrated. It is possible that adrenergic and muscarinic inputs to ciliary body help to regulate aqueous secretion,\(^\text{6,13}\) but the role for the large synergistic Ca\(^{2+}\) mobilization produced by simultaneous muscarinic and adrenergic activation and the mechanism of this activation remains to be elucidated. Because single NP cells reproduce the responses we have observed in intact tissue or NP layers, they should provide another tool with which to examine this response, in particular, and the physiology and pharmacology of ciliary body epithelium, in general.

References


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