Carbachol Increases Basolateral $K^+$ Conductance in T84 Cells

Simultaneous Measurements of Cell $[Ca]$ and $g_K$

Explore Calcium’s Role

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ABSTRACT To explore the role of calcium in mediating the action of carbachol in chloride-secreting epithelia, we simultaneously measured intracellular free $[Ca]$ ($[Ca]_i$) and the potassium conductance ($g_K$) of the basolateral membrane in T84 cells grown on collagen-coated filters. $[Ca]_i$ was measured with fura-2 and fluorescence microscopy and expressed as a relative value ($[Ca]_i/[]$ normalized to control. To assess changes in basolateral $g_K$, we measured the short circuit current ($I_{sc}$) in the presence of luminal amphotericin and a transepithelial mucosa-to-serosa $K^+$ gradient (Germann, W. J., M. E. Lowy, S. A. Ernst, and D. C. Dawson. 1986. J. Gen. Physiol. 88:237–251). Treatment of the monolayers with carbachol resulted in a parallel increase and then decrease in $[Ca]_i$ and $g_K$. The carbachol-induced changes in $g_K$ appeared to be dependent on the increase in $[Ca]_i$ because stimulation of $g_K$ was significantly diminished when the hormone-induced increase in $[Ca]_i$ was blunted, either by loading the cells with BAPTA or by reducing the extracellular $[Ca]$. The carbachol-stimulated increase in $g_K$ appeared to be the direct result of the increase in steady-state $[Ca]_i$. The changes in $g_K$ and $[Ca]_i$ after stimulation with carbachol were correlated and ionomycin also increased $g_K$ and $[Ca]_i$ in a parallel manner. The carbachol-induced $\Delta g_K$ per $\Delta [Ca]_i$, however, was greater than that after ionomycin. Because ionomycin and carbachol appear to open the same channel, a conclusion based on inhibitor and selectivity experiments, carbachol may have a second action that amplifies the effect of calcium on $g_K$.

INTRODUCTION

Intracellular free calcium ($[Ca]_i$) serves as one of carbachol’s second messengers in T84 cells, which secrete chloride from the basolateral to the luminal side (Dharmsathaphorn and Pandol, 1986; Dharmsathaphorn et al., 1989; Wong et al., 1989).

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Earlier studies (McRoberts et al., 1985; Dharmsathaphorn and Pandol, 1986) suggested that one of the sites of calcium's action is a potassium channel in the basolateral membrane. Both carbachol and the calcium ionophore ionomycin stimulated a quinidine-sensitive rubidium flux across the basolateral membrane.

The mechanism by which carbachol, acting via calcium, might increase the basolateral \( g_K \) in T84 cells could be similar to that demonstrated in exocrine cells (for reviews, see Petersen and Maruyama, 1984 and Petersen, 1986). In numerous tissues carbachol increases cell [Ca], which in turn opens large conductance calcium-activated channels in the basolateral membrane (Maruyama et al., 1983; Trautmann and Marty, 1984; Petersen and Findlay, 1987; Richards et al., 1989). These channels are sensitive to [Ca] in a range well within that attained during stimulation with carbachol.

Recent experiments suggest that the basolateral potassium channels in T84 cells are not likely to be of the large conductance variety and probably are of the small conductance class found in trachea (Welsh and McCann, 1985) and MDCK cells (Kolb et al., 1987). The carbachol-stimulated \( g_K \) in T84 cells is insensitive to both barium and TEA (Devor et al., 1990), quite unlike the large conductance channels (Latorre and Miller, 1983). Carbachol might nevertheless act via an increase in cell [Ca] because the carbachol and ionomycin-stimulated \( g_K \) have similar characteristics, indicating that \( g_K \) is calcium activated (Devor et al., 1990).

If carbachol increases \( g_K \) in T84 cells via a change in steady-state [Ca], as it does in exocrine cells, the range of [Ca] in which calcium-activated channels open should be similar to the range of cell [Ca] achieved after stimulation with carbachol. This comparison cannot be made in T84 cells because the calcium sensitivity of \( g_K \) is presently unknown. However, single channel calcium activation studies of the small conductance channel have been performed in other chloride-secreting epithelial tissues. In the trachea the channel remains in a closed state when [Ca] is 100 nM and opens submaximally when [Ca] is increased to 1 \( \mu \)M (Welsh and McCann, 1985), whereas in MDCK cells the channel is more sensitive to calcium, opening at 100 nM and reaching maximal conductance at 1 \( \mu \)M (Kolb et al., 1987). Given the fact that carbachol rarely increases [Ca], to levels significantly above 300 nM in T84 cells (Dharmsathaphorn et al., 1989; Wong et al., 1989), it would appear that the small conductance channel might remain unopened during carbachol's action.

Carbachol thus might not activate \( g_K \) via changes in cell [Ca] in T84 cells if the small conductance calcium-activated channel does not open at levels of [Ca] achieved during stimulation with carbachol. There are other explanations accounting for this discrepancy, however. First, the conditions under which calcium-activation curves and cell [Ca] are measured are quite different. Patch clamp studies are performed by exposing naked membrane to EGTA-calcium buffered solutions, whereas measurements of cell calcium with calcium-sensitive dyes are performed in intact cells. Thus, if the cytoplasm contained factors that increased the affinity of the channel for calcium, the affinity measured in an intact cell might be lower than that measured in a cell-detached patch of membrane. Second, carbachol might have another action that raises the affinity of the channel for calcium, such as phosphorylation (Ewald et al., 1985). Third, the action of calcium on the basolateral potassium conductance might be an indirect mechanism. The rise in cell [Ca] could trigger a
second event which in turn opens the channel. If this were the case, \( g_K \) might not be a function of the steady-state \([Ca]_i\).

There are understandable difficulties in using a comparison of calcium activation curves with measurements of cell \([Ca] \) to reach a conclusion regarding calcium’s role in regulating \( g_K \) in T84 cells. We sought a more direct approach to this problem and devised a method to make simultaneous measurements of intracellular \([Ca] \) and basolateral \( g_K \) (in the same tissue). Our results suggest that the action of carbachol on the basolateral \( g_K \) is mediated by changes in the steady-state \([Ca]_i\). Carbachol increases \( g_K \) and \([Ca]_i \) in a parallel manner throughout the effect of the hormone, the hormone-induced changes in \( g_K \) are correlated to the carbachol-induced changes in \([Ca]_i \), and the increase in \( g_K \) is dependent on the carbachol-induced increase in \([Ca]_i \). However, carbachol appears to be more effective than ionomycin at stimulating \( g_K \) per change in \([Ca]_i \), suggesting that carbachol might have a second action that enhances the effect of calcium on \( g_K \).

**METHODS**

**Cell Culture**

T84 cells, derived from human colonic cancer (Murakami and Masui, 1980), were cultured as described previously (Dharmsathaphorn et al., 1984; Wong et al., 1989). To measure \([Ca]_i \) and short circuit current \( (I_s) \), cells were plated on collagen-coated filters (Transwell filters; Costar, Cambridge, MA) at a density of \( 2.4 \times 10^6 \) cells/4.7 cm\(^2\). It took ~2 wk for the cells to reach confluence and develop a transepithelial resistance high enough to allow for measurements of \( I_s \) (>600 Ω · cm\(^{-2}\)).

**Measurement of \([Ca]_i \)**

\([Ca]_i \) was measured with fura-2 and fluorescence microscopy using methods described previously (Chase and Wong, 1988; Wong and Chase, 1988; Wong et al., 1989). In brief, cells grown on filters were removed from the incubator and bathed in Hanks buffer containing (in mmol/liter): 137 NaCl, 4.2 NaHCO\(_3\), 0.3 Na\(_2\)HPO\(_4\), 5.4 KCl, 0.4 KH\(_2\)PO\(_4\), 1.3 CaCl\(_2\), 0.5 MgCl\(_2\), 0.8 MgSO\(_4\), and 20 HEPES, brought to a pH of 7.5. The cells were loaded by incubation with a Hanks solution containing 10 μM fura-2 acetoxymethylester and 0.1% albumin on the serosal side. After loading was completed the cells were washed free of the ester by rinsing numerous times with Hanks buffer. Approximately 30 min later the cells were placed into the Ussing chamber on the stage of the microscope for fluorescence readings. When solutions were changed during an experiment, the lower or upper chamber was emptied using light suction and the solution was replaced with buffer. All experiments were performed at room temperature.

Epifluorescence was measured using an upright microscope (Carl Zeiss, Inc., Thornwood, NY) equipped with a 75-W xenon light source, a water immersion 40× lens, and a silicon intensifier target camera (SIT camera, series 66; Dage-MTI, Inc., Michigan City, IN). The sample was excited at 380 and 350 nm using band pass filters that were changed automatically. The image from the camera was recorded by a video recorder and displayed on a video monitor. The signal output from the video recorder first passed through a video analyzer (Instrumentation for Physiology & Medicine; San Diego, CA) before going to the monitor. The fluorescence of the sample was measured using the video analyzer, which transformed the light intensity of the image on the video screen to a voltage yielding a “real time” strip-chart record of the changes in light intensity.
Calculation of [Ca]$_i$

One can derive a value for [Ca], by calculating the fractional saturation of the dye. We used 380/350 nm ratios ($r$), rather than fluorescence at a single wavelength, to make this calculation (Grynkiewicz et al., 1985). Thus,

$$\frac{(r_{\text{max}} - r)}{(r_{\text{max}} - r_{\text{min}})} = \frac{1}{(K_d(s_f/s_b)/[Ca]) + 1} \quad (1)$$

where $r_{\text{max}}$ and $r_{\text{min}}$ are the maximum and minimum fluorescence 380/350 ratios and $s$ is the fluorescence at 350 nm when the dye is either free of calcium ($s_f$) or saturated with calcium ($s_b$). By arrangement

$$[Ca] = K'_d \frac{(r_{\text{max}} - r)}{(r - r_{\text{min}})} \quad (2)$$

where $K'_d = K_d \cdot s_f/s_b$.

To calculate [Ca], the maximum and minimum signals must be known to define the boundaries of dye fluorescence. With these ratios [Ca], is calculated by inserting the value for the $K'_d$ (160 nM, based on the published $K_d$ of 200 nM [Grynkiewicz et al., 1985] and the value of $s_f/s_b$ of 0.8, experimentally determined at 350 nm). To obtain the minimal fluorescence ratio, we ended each experiment by adding 5 µM ionomycin, a calcium ionophore, and excess calcium (~30 min). To obtain the maximal fluorescence we added excess EGTA to a calcium-free buffer. Using this protocol we determined in a previous publication (Wong et al., 1989) that the average resting [Ca], was $25 \pm 5$ nM ($n = 4$).

Normalization of [Ca]$_i$

In many experiments ionomycin was added to increase [Ca], to very high levels. Once [Ca], rose to these levels, however, it was often impossible to lower [Ca], with EGTA to obtain the maximal fluorescence ratio. Without a maximal value of fluorescence, a precise calculation of [Ca], could not be made. To present the data in a consistent manner we derived a normalized value of [Ca], ($[Ca]_n$) (Wong et al., 1989). This was accomplished by assigning a value for the missing maximum fluorescence so that at the start of each experiment the derived value of [Ca], was 25 nM, the average control [Ca], (see above).

Measurement of Potassium Conductance of the Basolateral Membrane

The basolateral potassium conductance was measured using the technique developed by Dawson and co-workers (Kirk and Dawson, 1983; Germann et al., 1986). A potassium gradient ($m-s$) is first established across the monolayer by substituting KCl for NaCl in the Hanks buffer bathing the mucosal side. Amphotericin is then added to the mucosal side to eliminate the mucosal barrier as rate limiting to transepithelial potassium movement. Under the conditions of the experiment, in which the monolayer is short circuited (i.e., voltage-clamped at zero potential) and the transepithelial potassium gradient is constant, the amphotericin-dependent $I_x$ becomes a measure of the rate of the transepithelial potassium flux. Because the flux of potassium across the cell is limited by the basolateral potassium conductance, a change in $I_x$ represents, in turn, a change in $g_K$.

$I_x$ was measured using calomel electrodes, 3 M KCl-agar bridges, and a DVC-1000 voltage clamp (World Precision Instruments, Inc., New Haven, CT). Resistance was measured by pulsing the tissue with enough current to increase the transepithelial potential 5 or 10 mV.

Use of Gluconate Buffers

In a number of experiments gluconate was substituted for chloride. Because of the significant chelation of calcium by gluconate, the total [Ca] of the gluconate–Hanks buffers had to be
increased to 5–7 mM so that the free [Ca] would be the same (1 mM) as the chloride-containing buffers. Free [Ca] was measured using a calcium electrode (Radiometer America, Inc., Westlake, OH).

Statistics

Results are expressed as the mean ± SEM. We used the Student's t test to compare different groups in individual experiments or the percent increase over control. A result was considered significant if \( P < 0.05 \). For each group of experiments \( n \) refers to the number of experiments performed.

![Graph showing effects of amphotericin and carbachol on \( I_c \) and [Ca]i.](image)

**Figure 1.** Effects of amphotericin and carbachol on \( I_c \) and [Ca]i. \( I_c \) and [Ca]i were measured simultaneously while monolayers were first exposed to a transmembrane potassium gradient and then treated with 10 \( \mu \)M amphotericin on the mucosal side. After ~40 min 100 \( \mu \)M carbachol was added to the serosal side. (Note change in time scale.)

**RESULTS**

*Effects of Amphotericin and Carbachol on [Ca]i and Potassium Conductance of the Basolateral Membrane*

A typical experiment, in which the transepithelial potassium flux (\( I_e \)) and [Ca]i were recorded simultaneously, is depicted in Fig. 1. First, a potassium gradient was established across the monolayer (m→s) resulting in a small change in \( I_e \) and no change in [Ca]i. After addition of amphotericin to the mucosal side there was a slow and steady increase in \( I_e \) and [Ca]i over 30 min. When 100 \( \mu \)M carbachol was added to the serosal side, there was a large and parallel increase in \( I_e \) and [Ca]i and then a parallel decline in both parameters. In four similar experiments carbachol caused \( I_e \) to increase from 161 ± 52 \( \mu \)A to a peak of 273 ± 88 and [Ca]i to rise from 104 ± 17
nM to a peak of 342 ± 74. Approximately 1–2 min after the peak response, both $I_c$ and $[Ca]_i$ returned to values slightly above the control value.

Fig. 1 also demonstrates that the addition of amphotericin unmasks a very large $g_K$. Previous work established that this large increase in $g_K$ is due to cell swelling, observed only when chloride is present in the buffers (Germann et al., 1986). To eliminate any effect that cell swelling might have on the cell's response to carbachol, we examined the effect of carbachol on $I_c$ and $[Ca]_i$ in chloride-free buffers. As shown in Fig. 2, the addition of amphotericin to the mucosal bathing solution had no effect on $I_c$ when chloride was absent from the buffers. When carbachol was then added to the serosal solution the same parallel increase in $I_c$ and $[Ca]_i$ occurred (Fig. 2 A). $I_c$ and $[Ca]_i$ first increased and decreased quickly and then declined further over the next 5 min. The responses of $I_c$ and $[Ca]_i$ to carbachol were reversed by washing the hormone from the basolateral compartment. Not only were the
carbachol-induced changes in $I_K$ and $[Ca]_i$ parallel, they were also correlated (Fig. 2B). In a plot of $I_K$ as a function of $[Ca]_i$, the control, carbachol peak, 2- and 5-min points, and wash all fall on the same line.

Dependence of the Carbachol-induced Changes in $g_K$ on the Increase in $[Ca]_i$

To determine if the carbachol-induced increase in $g_K$ was dependent on the rise in steady-state $[Ca]_i$, we examined the effect on $\Delta g_K$ of diminishing the hormone-

FIGURE 3. Effect of BAPTA loading on the response to carbachol. (A) Cells were loaded with BAPTA and then exposed to 100 µM serosal carbachol. (B) Results from a number of experiments were pooled to compare the response to carbachol of BAPTA-loaded and control monolayers. Buffers contained chloride.

stimulated increase in $[Ca]_i$. In the first series of experiments cells were loaded with the intracellular calcium buffer BAPTA (Tsien, 1980). As demonstrated in Fig. 3A, the effect of carbachol on $I_K$ and $[Ca]_i$ in BAPTA-loaded cells was delayed, and the peak increases in $I_K$ and $[Ca]_i$ were significantly blunted (compare with the control
response depicted in Fig. 1). The peak in $I_{\text{sc}}$ and $[\text{Ca}^+]_i$ did not occur for almost 2 min and the magnitude of the peak was well below the response in the control monolayers. The blunted response of the BAPTA-loaded cells is best demonstrated in a plot of $I_{\text{sc}}$ as a function of $[\text{Ca}^+]_i$ (Fig. 3 B), showing that the increases in $I_{\text{sc}}$ and $[\text{Ca}^+]_i$ were clearly not of the same magnitude as in the control cells.

We used a second procedure, lowering extracellular $[\text{Ca}]$, to reduce the magnitude of the carbachol-induced increase in $[\text{Ca}^+]_i$ (Penner et al., 1988). Fig. 4 A demonstrates the effect of lowering the extracellular free $[\text{Ca}]$ on the response to

![Graph A](image)

**Figure 4.** Relationship between $[\text{Ca}^+]_i$ and $g_K$. (A) Effect of extracellular $[\text{Ca}]$ on the response to carbachol in chloride-free solutions. Monolayers were bathed in either 100 μM or 1 mM free $[\text{Ca}]$ chloride-free buffer. A calcium electrode was used to measure free $[\text{Ca}]$. Results were expressed as a percent above the control values of $I_{\text{sc}}$ and $[\text{Ca}]$. In 1 mM $[\text{Ca}]$ control $I_{\text{sc}}$ and $[\text{Ca}]$ were 52 ± 8 μA and 97 ± 13 nM, respectively; at 100 μM $[\text{Ca}]$ $I_{\text{sc}}$ and $[\text{Ca}]$ were 46 ± 7 μA and 112 ± 14 nM, respectively. The points represent the mean of four experiments. (B) *Left.* The %Δ$I_{\text{sc}}$ was plotted as a function of %Δ$[\text{Ca}^+]_i$ using the peak, 2-min and 4-min values after stimulation with carbachol in low and high $[\text{Ca}]$. *Right.* The slope of the Hill plot was 2.99 ± 0.35 and $K_{1/2}$ was 238 nM. Using these values a sigmoidal curve was generated and displayed in the plot on the left.
carbachol. The responses of both $I_{sc}$ and $[Ca]_i$ (left and right panels, respectively) were blunted when extracellular free [Ca] was reduced to 100 μM, and only in the low [Ca] buffer did $I_{sc}$ and $[Ca]_i$ return to control levels after 4 min. When these data were replotted to examine the relationship between Δ$I_{sc}$ and Δ$[Ca]_i$, it was apparent that changes in $I_{sc}$ were tightly correlated to changes in the steady-state $[Ca]_i$ (Fig. 4 B, left). A Hill analysis (Fig. 4 B, right) of these data generated a slope of ~3,

![Graph A](image)

**Figure 5.** Effect of ionomycin and then carbachol on $I_{sc}$ and $[Ca]_i$. (A) At the start of the trace amphotericin was added while $I_{sc}$ and $[Ca]_i$ were measured. 1 μM ionomycin was then added to the serosal side. While the ionophore was still present, 100 μM carbachol, dissolved in an ionomycin-containing buffer, was added to the serosal side. (B) The relationship between the steady-state $I_{sc}$ and $[Ca]_i$ is demonstrated. The time sequence of the points move from left to right. In three similar experiments the slopes were 0.6 ± 0.2 and 1.3 ± 0.2, respectively ($P < 0.05$).

indicating that the relationship between the changes in $g_K$ and $[Ca]_i$ was cooperative and, as a minimum, three calcium ions were necessary to activate $g_K$.

To distinguish direct effects of calcium on $g_K$ from other possible actions of carbachol, we determined if the calcium ionophore, ionomycin, also acted to increase $g_K$ and $[Ca]_i$. Fig. 5 A demonstrates that ionomycin, like carbachol, caused $I_{sc}$ and $[Ca]_i$ to increase in parallel. However, when carbachol was added $I_{sc}$ still
increased significantly, even after it had already risen to a very high value. Although 
$[\text{Ca}]_i$ also increased further, the carbachol-induced change in $I_c$ per change in $[\text{Ca}]_i$ 
($\Delta I_c/\Delta [\text{Ca}]_i$) was much greater than that observed after ionomycin. The dissimilar 
effects of carbachol and ionomycin on $\Delta I_c/\Delta [\text{Ca}]_i$ are best demonstrated in Fig. 5B, 
where it is shown that the slope of the relationship ($\Delta I_c/\Delta [\text{Ca}]_i$) is steeper after 
carbachol than after ionomycin. A similar result was obtained if we reversed the 
order of the experiment: first adding carbachol, washing, and then adding ionomycin 
(Fig. 6).

![Graph](image)

**Figure 6.** Effect of carbachol and ionomycin on $I_c$ and $[\text{Ca}]_i$. (A) A typical tracing 
demonstrates the relationship between $\Delta I_c$ and $[\text{Ca}]_i$ during stimulation with carbachol and 
ionomycin. After monolayers were treated with amphotericin (start of trace) carbachol was 
added in graded doses (10 and 100 $\mu$M). Carbachol was then washed off and replaced with 1 
$\mu$M ionomycin. (B) Steady-state values of $I_c$ and $[\text{Ca}]_i$ were plotted. There are five carbachol 
points, representing the peak values at 10 and 100 $\mu$M and the control and wash points. The 
value obtained after washing before ionomycin is indicated by an asterisk. The ionomycin 
values, indicated by the squares, follow sequentially as the line moves from left to right.

The quantitative dissimilarity between the effects of carbachol and ionomycin 
suggested that carbachol might open a second channel in addition to the calcium-
activated channel also opened by ionomycin. To explore this issue we examined the 
inhibitor profile and ion selectivity of the carbachol and ionomycin-stimulated $g_K$. As 
shown in Table I, the hormone- and ionomycin-induced $g_K$ had the same inhibitor
sensitivity and ion selectivity, clearly different from the $g_K$ observed after the addition of amphotericin alone: it was blocked by quinidine, was resistant to both barium and TEA, and permitted rubidium to pass through as easily as potassium. Carbachol and ionomycin are thus likely to activate the same basolateral potassium channel.

**DISCUSSION**

*Role of Calcium in the Action of Carbachol on the Basolateral Potassium Conductance*

The carbachol-activated changes in basolateral $g_K$ in T84 cells are probably due to the changes in the steady-state $[Ca]_i$. This conclusion follows from the observations that the hormone-induced changes in $g_K$ and $[Ca]_i$ are parallel throughout the entire effect of the hormone (Fig. 2B), that the increase in $g_K$ is correlated to the rise in $[Ca]_i$ (Figs. 3 and 4B), and that increasing cell $[Ca]$ with ionomycin increases $[Ca]_i$.

**Table I**

| $\Delta I_{il}$ induced by | Percent inhibition of $\Delta I_{il}$ | Selectivity |
|---------------------------|----------------------------------------|-------------|
|                           | Barium | Quinidine | TEA | $\Delta I_{il} (Rb^+/K^+)$ |
| 10 µM amphotericin        | 74 ± 3 | 79 ± 2    | −0  | 0.26 ± 0.02 |
| 100 µM carbachol          | −0    | 78 ± 2    | −0  | 0.28 ± 0.04 |
| 5 µM ionomycin            | −0    | 74 ± 19   | −0  | 1.35 |

All experiments were performed in Hanks buffer with chloride. In each group at least four experiments were performed. 5 mM barium and 10 mM TEA were added to the serosa while 250 µM quinidine was added to the mucosa.

*Though TEA actually inhibited the response to carbachol by 42 ± 8%, the response was probably due to the atropine-like action of TEA (Sutliff et al., 1989). Using a $K_t$ for TEA of 1.4 mM and a $K_t$ for carbachol of 170 µM, 10 mM TEA would be expected to inhibit the action of 100 µM carbachol by 52%. Thus, the effect of TEA on carbachol's stimulation of $g_K$ was negligible. *Not significantly different from 1.0. †This value was determined by dividing the ionomycin-induced $\Delta I_{il}$ using rubidium (179 ± 19 µA) by $\Delta I_{il}$ using potassium (135 ± 27). There is no significant difference between these two means.

and $g_K$ in a parallel manner (Fig. 5A) by opening the same channel as that activated by carbachol.

Calcium thus appears to serve as carbachol's second messenger in T84 cells, as it does in exocrine tissues (Maruyama et al., 1983; Trautmann and Marty, 1984; Petersen and Findlay, 1987; Richards et al., 1989). This is an interesting observation considering that the basolateral channel activated in T84 cells is more likely to be of the small conductance variety, identified in both epithelial and nonepithelial tissues (Welsh and McCann, 1985; Sauve et al., 1986, 1987; Kolb et al., 1987; Furuya et al., 1989; Gallin, 1989), than of the large conductance type found in exocrine cells (Petersen, 1986). This conclusion is based on the observations that the calcium-activated $g_K$ in T84 cells (our studies and Devor et al., 1990) is barium and TEA insensitive, and, like the barium- and TEA-insensitive basolateral channel in turtle colon (Chang and Dawson, 1988), conductive to rubidium. This particular inhibitor and selectivity profile is uncharacteristic of the large conductance channel (Latorre
and Miller, 1983) and more representative of the small conductance channel which is resistant to barium (Gallin, 1989) and TEA (Furuya et al., 1989). Demonstration that the basolateral calcium-activated potassium channel in T84 cells is of the small conductance variety will require single channel studies.

The mechanism by which calcium increases $g_K$ remains unknown. The action could be a direct one in which calcium interacts with the channel to open it, or an indirect one in which calcium associates with an intermediary to open the channel. For example, calmodulin could serve as a middleman as it does in Paramecium (Hinrichsen et al., 1986). Future studies should be designed to elucidate the precise mechanism by which calcium increases $g_K$.

**Does Carbachol Act Exclusively via Changes in Cell [Ca] to Increase $g_K$?**

Simultaneous measurements of $g_K$ and [Ca]$_i$ demonstrate that during the action of both carbachol and ionomycin changes in $g_K$ are correlated to changes in the steady-state [Ca]$_i$. However, the experiments in Figs. 5 and 6 demonstrate that during carbachol’s action the relationship between the changes in $g_K$ and [Ca]$_i$ ($\Delta g_K/\Delta$[Ca]$_i$) is significantly greater than during ionomycin’s effect. These observations are consistent with the view that carbachol has two actions, the first to increase [Ca]$_i$ and the second to amplify calcium’s effect on $g_K$. Further support of this idea is demonstrated in Fig. 5, showing that carbachol significantly increases $g_K$ even after [Ca]$_i$ had risen to very high levels, and does so without substantially increasing [Ca]$_i$.

If carbachol stimulates a second process to enhance calcium’s effect on basolateral $g_K$, it could do so in one of a variety of ways, including activation of a calcium-dependent kinase such as protein kinase C (Audigier et al., 1988) or the calcium-calmodulin dependent kinase (Connelly et al., 1987), stimulation of exocytosis and insertion of calcium-activated potassium channels into the basolateral membrane (van Adelsberg and Al-Awqati, 1986), or increasing cell pH (Melvin et al., 1988) making $g_K$ more sensitive to changes in calcium (Christensen and Zeuthen, 1987).

Before concluding that carbachol has a second action, however, it must be demonstrated that the apparent dissimilarity of the carbachol- and ionomycin-induced $\Delta g_K/\Delta$[Ca]$_i$ is genuine. It is possible that the carbachol-stimulated increase in [Ca]$_i$ is predominantly in the vicinity of the basolateral membrane, while the increase in [Ca]$_i$ after ionomycin is more evenly distributed throughout the cell. A similar conclusion was reached in studies of parotid acinar cells in which it was shown that though the carbachol-stimulated $g_K$ was dependent on a rise in [Ca]$_i$, activation of $g_K$ could be observed before any detectable change in [Ca]$_i$. (Foskett et al., 1989). It seemed as if the true [Ca]$_i$ in the vicinity of the basolateral channel was much higher than that recorded from whole cell fluorescence measurements.

Using our present methodology, in which fluorescence readings represent an average value throughout the whole cell, we are unable to ascertain if the carbachol-

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In previous studies of T84 cells and sweat glands (Sato and Sato, 1988; Dharmsathaphorn et al., 1989; Wong et al., 1989) carbachol was found to be more effective than ionomycin in stimulating secretion. However, because secretion is a multi-step process, carbachol’s superior action could be explained by a calcium-activated action at one step and a calcium-independent action at another. Our present results suggest, however, that there may be two carbachol-stimulated actions converging on a final common pathway.
induced \( \Delta I_K/\Delta [Ca] \) is the same or greater than that after ionomycin. Studies using confocal fluorescence microscopy will be required to determine directly if there are differences in the spatial localization of changes in [Ca], after ionomycin and carbachol. If \( \Delta I_K/\Delta [Ca] \) in the vicinity of the potassium channel after the two agents are indeed the same, then it will not be necessary to invoke a second carbachol-activated process.

One other explanation of the dissimilar response of the cells to ionomycin and carbachol is that ionomycin has a second, inhibitory action which reduces the sensitivity of \( g_K \) for calcium. This is a less likely reason because carbachol increased \( g_K \) in ionomycin-treated cells to a degree comparable to that observed in the non-ionomycin-treated control cells (Fig. 5).

**Role of the Calcium-activated \( g_K \) in Transepithelial Chloride Secretion**

Previous studies have shown the carbachol-induced increase in chloride secretion in T84 cells is insensitive to both barium and TEA and inhibited by quinidine (Dharmsathaphorn and Pandol, 1986). The fact that the inhibitor profile is identical to that of the carbachol-stimulated \( g_K \) suggests that hormone activation of \( g_K \) is essential in the hormone-activated increase in transepithelial chloride secretion.

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