Type II protein kinase A regulates CFTR in airway, pancreatic, and intestinal cells

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Type II protein kinase A regulates CFTR in airway, pancreatic, and intestinal cells. Am. J. Physiol. 274 (Cell Physiol. 43): C819–C826, 1998.—The type of protein kinase A (PKA) responsible for cystic fibrosis transmembrane conductance regulator (CFTR) activation was determined with adenosine 3',5'-cyclic monophosphate analogs capable of selectively activating type I or type II PKA. The type II-selective pair stimulated chloride efflux in airway, pancreatic, and colonic epithelial cells; the type I-selective pair only stimulated a calcium-dependent efflux in airway cells. The type II-selective analogs activated larger increases in CFTR-mediated current than did the type I-selective analogs. Measurement of soluble PKA activity demonstrated similar levels stimulated by type I- and type II-selective analogs, creating an apparent paradox regarding the outcome of PKA activation. Rat parotid gland secretion measured after the type I-selective analogs resulted in an increase in current; little increase was seen after the type II-selective analogs. Measurement of insoluble PKA activity stimulated by the analogs resolved this paradox. Type II-selective analogs stimulated three times as much insoluble PKA activity as the type I-selective pair, indicating that differential activation of PKA in cellular compartments is important in CFTR regulation.

Cystic fibrosis; adenosine 3',5'-cyclic monophosphate; ion channels; cystic fibrosis transmembrane conductance regulator

THE CYSTIC FIBROSIS transmembrane conductance regulator (CFTR) is an adenosine 3',5'-cyclic monophosphate (cAMP)-regulated chloride channel activated by phosphorylation by protein kinase A (PKA). CFTR is expressed in a variety of tissues, including respiratory epithelium, sweat and pancreatic glands, and intestinal epithelial cells. Mutations in CFTR may lead to cystic fibrosis, a recessive autosomal lethal disease characterized by progressive respiratory failure and infection, exocrine pancreatic dysfunction, gastrointestinal blockage, high sweat chloride concentrations, and infertility (29). Regulation of CFTR-mediated chloride permeability is achieved through two events: phosphorylation of multiple serines in the R, or regulatory, domain of the protein, followed by binding and hydrolysis of ATP at the nucleotide-binding folds (5, 12, 14). It has been shown that some mutant CFTR channels can be activated pharmacologically by compounds that stimulate or inhibit components of the PKA pathway such as β-adrenergic receptors, adenylyl cyclase, phosphodiesterases, phosphatases, and PKA itself (3, 10, 15, 16). The existence of several isoforms of each component, however, adds complexity to determining which pharmacological agent could stimulate or inhibit this pathway. To more completely understand the regulation of CFTR and to develop more specific methods of pharmacological activation of CFTR mutants, we characterized the type of PKA involved in CFTR regulation in cell lines derived from three of the tissues most clinically affected in cystic fibrosis: airway, pancreas, and intestines.

PKA is a holoenzyme consisting of a regulatory subunit dimer and two catalytic subunits. Each regulatory subunit contains two sites for cAMP binding; each site has different affinities for cAMP and acts cooperatively to bind cAMP. On binding of four molecules of cAMP, the affinity of the regulatory subunits for the catalytic subunits decreases 10,000- to 100,000-fold, and the catalytic subunits are released and may phosphorylate specific protein targets (reviewed in Refs. 11 and 27). PKA exists as two types, I and II, which are defined by the type of regulatory subunit present in the holoenzyme, RI and RII, respectively. The regulatory subunits differ in molecular weight, protein sequence, phosphorylation state, tissue distribution, subcellular location, and other biochemical properties (11, 27). The RII subunits have been found to be associated with A kinaseanchoring proteins (AKAPs), which may sequester the type II PKA in specific cellular compartments (7, 20). The localization of types may serve to enhance the specificity of the PKA reaction by compartmentalization of PKA with preferred substrates. Isoforms of each of the regulatory subunits have been found (RIα, RIβ, RIIα, and RIIβ, with the α-isofoms being expressed in most cells; there are also three types of catalytic subunits (Ca, Cβ, and Cγ)). The catalytic subunits are found interchangeably with the regulatory homodimers (11, 27).

To better understand the activation pathway of CFTR, we designed experiments to determine which type of PKA regulates CFTR in airway, pancreatic, and intestinal cells. CAMP analogs that preferentially activate a specific type of PKA were used in assays designed to measure CFTR-mediated chloride secretion. These CAMP analogs not only have high stability and membrane permeability, they also have selective binding affinities for the A or B cAMP-binding sites of the regulatory subunit isoforms (RI and RII) (reviewed in Ref. 4). By combining pairs of analogs that are selective for A and B sites of a particular PKA type, one can preferentially activate that type and measure a specific outcome of PKA activation. Rat parotid gland secretion (13, 23), inhibition of T lymphocyte proliferation (26), steroidogenesis in rat adrenal (30) and Leydig cells (21), and growth inhibition of transformed cancer cells (6, 24) are just some of the phenomena that have been examined through the use of site-selective analogs to...
determine the type of PKA involved in each process. It is hoped that, by identifying the type of PKA responsible for CFTR regulation, pharmaceutical tools to activate mutant CFTRs can be developed.

MATERIALS AND METHODS

Drugs. 8-(6-Aminohexyl)aminoadenosine 3',5'-cyclic monophosphate (AHA-cAMP), 8-(4-chlorophenylthio)-adenosine 3',5'-cyclic monophosphate (CPT-cAMP), N6-benzoyladenosine 3',5'-cyclic monophosphate (N6-benzoyl-cAMP), forskolin, 4,4'-disothiocyostilbene-2,2'-disulfonic acid (DIDS), glibenclamide, and bumetanide were obtained from Sigma Chemical, 8-piperidinoadenosine 3',5'-cyclic monophosphate (PIP-cAMP) from BioLogs, and 1,2-bis(2-aminophenoxy)-ethane-N,N,N',N'-tetraacetic acid-acetyoxymethyl ester (BAPTA-AM) and nystatin from Calbiochem.

Cell culture. Calu-3 cells (25) were grown in Eagle's minimal essential medium (MEM) with Earle's balanced salt solution supplemented with sodium pyruvate, nonessential amino acids, 10% fetal bovine serum, and L-glutamate; T84 cells (1) in a 1:1 mix of Ham's F-12 and Dulbecco's minimal essential medium (DMEM) plus 5% fetal bovine serum, and L-glutamate; and Capan-1 cells (2, 17) in RPMI 1640 medium, 15% fetal bovine serum, and L-glutamate; all were aerated with 5% CO2 at 37°C. All cell lines were obtained from the American Type Culture Collection (Rockville, MD). Cells were seeded at a density of 1–5 x 10^5 cells/cm2. For isotopic efflux assays, cells were grown to confluency in six-well plates; cells for Ussing chamber experiments were grown to confluency on collagen-coated Millicell-CM permeable supports (0.63 cm2) from Millipore. Capan-1 and T84 cells were cultured on plastic before PKA activity measurements.

Isotopic efflux assays. The chloride efflux assay is a modification of that described by Venglarik et al. (28), in which chloride is substituted for iodide. Cells were incubated for 1.5 h in N2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES)-buffered Ringer solution (HBR) consisting of (in mM): 10 HEPES (pH 7.4), 138 NaCl, 5 KCl, 2.5 Na2HPO4, 1.8 CaCl2, 1 MgSO4, and 10 glucose (pH 7.4) on the apical side. All experiments were carried out at 37°C, and KBR was continuously bubbled with 95% O2-5% CO2 by air-lift circulators. Voltage damping was accomplished with a voltage-current clamp (model DVC 1000, WPI, Sarasota, FL). Transepithelial voltage (Vt) was recorded through agar bridges (4% agar in KBR) connected to balanced calomel electrodes. Vt was clamped to 0, and Isc was recorded through Ag-AgCl electrodes. Transepithelial resistance was calculated by measuring the change in Isc (An) in response to a +2-mV clamp of Vt (3-s pulse every 30 or 40 s) and was corrected for solution resistance. Data were collected on a MacLab/4e (AD Instruments, Milford, MA). In experiments where the basolateral membrane was permeabilized, nystatin (0.4–0.6 mg/ml) was added to the basolateral side 10–30 min before the addition of type II-selective cAMP analogs. Average resistances across cell monolayers were 288, 66, and 102 Ω·cm2 for Capan-1, T84, and Calu-3 cells, respectively.

Assay for activated PKA. Capan-1 or T84 cells were grown to confluency on plastic dishes and then assayed. Cells were incubated at 37°C for 15 min with AHA-cAMP plus PIP-cAMP (50 µM each analog), then washed, lysed in extraction buffer [5 mM EDTA, 50 mM tris(hydroxymethyl)aminomethane, pH 7.5], and spun for 5 min at 14,000 g in a microfuge. The supernatant was kept as the soluble fraction, and the pellet was resuspended in extraction buffer plus 1% Triton X-100 and designated the particulate fraction. PKA activity was measured according to the manufacturer's directions (PKA assay system, GibCO BRL). Briefly, for each condition, four reactions were incubated for 5 min at 25°C with an oligopeptide (kemptide) as the substrate. Activity of the lysate less activity of the lysate in the presence of the PKA inhibitor gave the PKA activity of the fraction. Activity of the lysate in the presence of 4 µM cAMP less activity in 4 µM CAMP plus the PKA inhibitor gave the total PKA activity capable of being stimulated in that fraction of the cell. Pilot experiments were carried out to determine the optimal amount of lysate necessary to keep the rate of kemptide phosphorylation in the linear range. The amount of PKA activated by the analogs was calculated as the percentage of PKA activated relative to the total cAMP-activatable PKA activity in that fraction.

Statistics. Paired comparisons were evaluated by t-test. Significance was arbitrarily chosen as α = 0.05.

RESULTS

We investigated which type of PKA activated CFTR in cell lines used as models for three tissues affected in cystic fibrosis: Capan-1, a human pancreatic adenocarcinoma cell line; T84, a human colon carcinoma cell line; and Calu-3, a human airway epithelial cell line. CFTR function was measured after incubation of the cells with combinations of cAMP analogs selective for type I or type II PKA. The combination of AHA-cAMP and PIP-cAMP was used to preferentially activate type I PKA, inasmuch as AHA-cAMP is selective for the B site of RI, whereas PIP-cAMP selectively binds the A site of RI and the B site of type II. The analog pair CPT-cAMP (selective for the B site of RII) and N6-benzoyl-cAMP (selective for the A site of either PKA isozyme) was used to preferentially activate type II PKA (4). For the following experiments, each analog was used at 50 µM, inasmuch as this concentration is submaximal in eliciting changes in Isc (data not shown) yet capable of stimulating PKA activity.
Stimulation of chloride efflux by PKA type-selective analog pairs. To compare the type of PKA activating CFTR in airway, intestinal, and pancreatic cells, isotopic efflux assays were performed on these cells in the presence of type-selective cAMP analog pairs. Capan-1, T84, and Calu-3 cells were grown to confluency and equilibrated in 36Cl-containing HBR. After cells were washed to remove extracellular chloride, the analog pairs were added to chloride-free HBR (50 µM each analog), and aliquots were replaced every 30 s to measure chloride efflux. For comparison, responses to the analogs were quantitated by averaging the efflux rates calculated for each 30-s time point over the 5 min in which the analogs were present. In these experiments we chose concentrations of cAMP analogs similar to those used in the literature (9, 26, 30). The type II-selective pair, CPT-cAMP plus N6-benzoyl-cAMP, stimulated a sustained chloride efflux in all three cell types (Fig. 1, Table 1). The type I-selective analog pair, AHA-cAMP plus 8-PIP-cAMP, resulted in a quick pulse of chloride secretion in Calu-3 cells that is transiently larger than that seen with the type II-selective analogs but leads to a smaller average rate of efflux over the time period measured. The type I-selective analogs did not stimulate significant efflux in T84 or Capan-1 cells. Therefore, the type II-selective analog pair stimulated a larger chloride efflux than the type I-selective pair in Capan-1 and T84 cells, but not in Calu-3 cells. Incubation of Calu-3 cells with the calcium chelator BAPTA-AM before addition of the analogs blocked the efflux stimulated by the type I-selective analog pair but had no effect on that stimulated by the type II-selective pair (Fig. 2). Similarly, BAPTA-AM had no effect on the effluxes stimulated by the type II-selective analogs in T84 or Capan-1 cells. These results showed that the chloride efflux activated by the type I-selective analogs was calcium dependent in Calu-3 cells, whereas that activated by the type II-selective analogs was not. The efflux stimulated by the type II-selective analogs may even be inhibited by calcium, inasmuch as there was a marked increase in this efflux in the presence of BAPTA-AM in Capan-1 and T84 cells. The results suggested that PKA II may activate a sustained, calcium-independent chloride efflux in Capan-1, T84, and Calu-3 cells, consistent with the activation of CFTR.

Stimulation of changes in Isc by analog pairs. To compare efflux results with electrogenic chloride transport, we also examined CFTR function by measuring the effect of the analogs on Isc across monolayers of the three cell types. Capan-1, T84, and Calu-3 cells were grown to confluency on collagen-coated filters and mounted in modified Ussing chambers. Chloride-free Ringer solution was placed on the apical side of the filters to increase the driving force for transepithelial chloride transport. Initial experiments were performed to determine the concentration of type-selective analogs that had an effect on Isc but did not maximally stimulate the current (data not shown), and as in the isotopic efflux assays, each analog was used at 50 µM. Addition of type I-selective analogs, AHA-cAMP plus PIP-cAMP, to the basolateral side of the cells resulted in minor increases in Isc (Table 2). When the type II-selective analogs CPT-cAMP and N6-benzoyl-cAMP were added, 3- to 30-times larger currents were stimulated, depending on the cell type. Addition of the Na-K-2Cl cotransporter blocker bumetanide (100 µM) to the basolateral side of the cells resulted in minimal increases in Isc, consistent with current due to chloride secretion. In each cell type the type II-selective analogs stimulated a significantly larger increase in Isc than did the type I-selective analogs (P < 0.05). Interestingly, the type I-selective analogs did not activate a large, transient increase in current analogous to the large, transient increase in chloride efflux seen in Calu-3 cells, suggesting that the increase in chloride efflux was not electrogenic or may be due to differences in the culturing conditions of the cells for the two types of experiments.

Inhibitor studies of the stimulation of Isc in Capan-1 cells. Our experiments showed that the type II-selective analogs stimulated a sustained calcium-

Table 1. Effect of type-selective analogs on average rate of 36Cl- efflux during stimulation in Capan-1, T84, and Calu-3 cells

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Type I</th>
<th>Type II</th>
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<tbody>
<tr>
<td>Avg efflux rate</td>
<td>n</td>
<td>Avg efflux rate</td>
<td>n</td>
</tr>
<tr>
<td>Capan-1</td>
<td>5.7 ± 0.3</td>
<td>3</td>
<td>6.3 ± 0.3</td>
</tr>
<tr>
<td>T84</td>
<td>2.9 ± 0.3</td>
<td>3</td>
<td>3.6 ± 0.2</td>
</tr>
<tr>
<td>Calu-3</td>
<td>4.4 ± 0.5</td>
<td>3</td>
<td>10.1 ± 0.9</td>
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Values are means ± SE expressed as percent Cl- released/30 s; n, number of experiments. Control, efflux in HEPES-buffered Ringer solution alone; type I, type I-selective analogs; type II, type II-selective analogs; analogs at 50 µM each.

Fig. 1. Effect of protein kinase A (PKA) type-selective analog pairs on 36Cl- efflux from Capan-1 (A), Calu-3 (B), and T84 (C) cells. Cells were loaded with 36Cl- and washed, and aliquots of buffer were taken and replaced every 30 s to measure Cl- efflux. Horizontal bars indicate presence of type I- or type II-selective analog pairs at 50 µM each analog. Efflux was calculated as percentage of Cl- secreted into buffer since previous time point and plotted as a function of time. Error bars, SE (n = 3).
independent chloride efflux and activated larger changes in $I_{sc}$ than the type I-selective analogs in Capan-1, T84, and Calu-3 cells. To determine whether the analogs were activating current consistent with CFTR, Capan-1 cells were incubated with the chloride channel blockers DIDS or glibenclamide before addition of the analogs. The amount of current stimulated by the type I-selective analogs was small, and incubation with 500 µM DIDS had an insignificant effect on current ($P = 0.16$). Incubation with DIDS also did not significantly affect current stimulated by the type II-selective analogs ($P = 0.22$; Table 3). In contrast, incubation of Capan-1 cells with 500 µM glibenclamide before analog addition significantly decreased the current generated by type I- or type II-selective analogs. The inhibition of current by glibenclamide but not by DIDS was consistent with the activation of current by the type II-selective analog pair through CFTR.

**Effect of nystatin permeabilization of the basolateral membrane in T84 cells.** Although PKA activates CFTR chloride channels, an alternative explanation of the above data was that the compounds used can affect basolateral potassium channels, thereby influencing the driving force for chloride secretion (1). To address this possibility, the basolateral membranes were permeabilized, and the above experiments were repeated. T84 cells were mounted in modified Ussing chambers and incubated for 10–30 min with 0.4–0.6 mg/ml nystatin applied to the basolateral side. Type II-selective analogs were then added, and the change in $I_{sc}$ was measured. There was no significant difference in current stimulated by the type II-selective analogs between the nystatin-treated and control T84 cells (Table 4). Bumetanide (100 µM) no longer decreased the current generated by the analogs in the nystatin-treated cells, indicating that the basolateral membrane had indeed been permeabilized. These experiments, in which ion channels in the apical membrane were assayed directly, suggested that type II-selective analogs stimulated current through an apical chloride channel, presumably CFTR, in T84 cells.

**Fig. 2.** Effect of calcium chelator 1,2-bis(2-aminophenoxy)ethane-N, N', N'-tetraacetic acid acetoxymethyl ester (BAPTA-AM) on cAMP analog-stimulated Cl⁻ efflux in Calu-3 (A and B), Capan-1 (C), and T84 (D) cells. Cells were incubated with 10 µM BAPTA-AM for 30 min before assay. Horizontal bars indicate presence of type-selective analogs at 50 µM each analog. Error bars, SE ($n = 3$). •, Type I-selective analogs; ○, type II-selective analogs; ▲, type I-selective analogs + BAPTA-AM; △, type II-selective analogs + BAPTA-AM; □, control.

| Table 2. Effect of type-selective cAMP analogs on $I_{sc}$ in Capan-1, T84, and Calu-3 cells |
|---------------------------------------------|----------------|----------------|
| Type I $\Delta I_{sc}$                     | Type II $\Delta I_{sc}$ |
| Capan-1 6.2 ± 2.7                         | 22.4 ± 6.4     |
| T84    0.3 ± 0.3                           | 9.7 ± 3.2      |
| Calu-3  7.7 ± 3.3                          | 52.6 ± 15.6    |

Values are means ± SE expressed as µA/cm²; $n$, number of experiments. $\Delta I_{sc}$, change in short-circuit current stimulated by cAMP analogs; analogs at 50 µM each.

| Table 3. Effect of chloride channel blockers on $\Delta I_{sc}$ stimulated by type I- or type II-selective analogs in Capan-1 cells |
|---------------------------------------------|----------------|----------------|
| Type I $\Delta I_{sc}$                     | Type II $\Delta I_{sc}$ |
| Untreated 6.2 ± 2.7                         | 22.4 ± 6.4     |
| DIDS treated 3.1 ± 0.7                      | 29.0 ± 4.5     |
| Glibenclamide treated 0.6 ± 0.3             | 6.1 ± 0.8      |

Values are means ± SE expressed as µA/cm²; $n$, number of experiments. Analogs at 50 µM each; DIDS and glibenclamide at 500 µM.
Table 4. Effect of permeabilization of basolateral membrane of T84 cells on ΔIsc stimulated by type II-selective analogs

<table>
<thead>
<tr>
<th></th>
<th>Type II</th>
<th>Bumetanide</th>
<th>n</th>
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<tbody>
<tr>
<td>Untreated</td>
<td>9.7 ± 3.2</td>
<td>−10.7 ± 3.4</td>
<td>11</td>
</tr>
<tr>
<td>Nystatin treated</td>
<td>8.5 ± 1.6</td>
<td>−11.1 ± 0.4</td>
<td>4</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed as μA/cm²; n, number of experiments. Type II-selective analogs were used at 50 μM each; bumetanide at 100 μM, and nystatin at 0.4–0.6 mg/ml.

Activation of PKA by type-selective analog pairs. The differences seen with regard to stimulation of CFTR with type I- vs. type II-selective analogs could be explained by the relative effectiveness of the analogs to activate PKA, rather than a specific ability to stimulate the different types of PKA. Capan-1 and T84 cells were incubated with type I- or type II-selective analog pairs (50 μM each analog, the same concentration of analogs used in the measurement of ΔIsc) for 15 min. Cells were washed and lysed, and the percentage of soluble PKA activated by the analogs was measured using the PKA assay system (see MATERIALS AND METHODS). Figure 3 shows the percentage of PKA activated by the analogs (i.e., amount activated/total activity in the soluble fraction). Type I- and type II-selective analog pairs activated soluble PKA to a similar level in both cell types. These results suggested that the difference in the ability of the type I- vs. type II-selective analogs to stimulate CFTR was due to the inability of the type I-selective analog pair to stimulate soluble PKA.

Effect of forskolin addition after type-selective analog pairs on ΔIsc. An apparent paradox arose, in that the quantities of type I- and type II-selective analogs inducing similar levels of PKA activity (Fig. 3) stimulated significantly different amounts of ΔIsc (Table 2). ΔIsc was again measured with the addition of 10 μM forskolin after the type I- or type II-selective analog pairs. Representative traces are shown in Fig. 4A. In all three cell lines, addition of forskolin increased ΔIsc after type I-selective analogs but had little additional effect on current when added after the type II-selective analogs. The percentage of ΔIsc due to the type-selective analogs compared with the total change in ΔIsc (cAMP analogs plus forskolin) was calculated (Fig. 4B). Type II-selective analogs stimulated a significantly larger proportion of maximal current than type I-selective analogs (P < 0.001) in all three cell types. These results suggested that the type II-selective analogs and forskolin were acting through the same pathway to stimulate CFTR-mediated current, yet the paradox of how forskolin stimulated more PKA activity and increased current in cells pretreated with the type I-selective analog pair remained.

Activation of insoluble PKA by type-selective analog pairs. The above data could be explained by differential activation of PKA in cellular compartments, inasmuch as type II PKA can be targeted to specific intracellular sites by proteins such as AKAPs. To test this possibility, activation of insoluble PKA activity by the type I- vs. type II-selective analogs was examined. Capan-1 cells were incubated with type I- or type II-selective analogs, washed, lysed, and fractionated into soluble and insoluble compartments. The percentage of PKA activated by the analogs was then measured with the PKA assay system, as described above. Although type I- and type II-selective pairs activated ~100% of the soluble PKA (Fig. 3), the type II-selective analogs stimulated approximately three times as much insoluble PKA activity as did the type I-selective analogs (23.9 ± 16.3% in unstimulated cells, 22.1 ± 6.5% in type I-stimulated cells, and 76.4 ± 14.0% in type I-stimulated cells, n = 3–4, P < 0.05 for type I vs. type II; Fig. 5). The amount of cAMP-activatable PKA was ~10% less in the insoluble fraction than in the soluble fraction, making the amount of insoluble PKA stimulated by the type II-selective analogs small compared with the total amount of PKA that can be activated in the whole cell (6.8% of the cellular PKA was stimulated by the type II-selective analogs in the particulate fraction, whereas the type I-selective analogs stimulated only 1.7%). Therefore, the type I-selective analogs stimulated 100% of soluble PKA activity but none of the particulate, whereas the type II-selective analogs stimulated PKA activity in the soluble and insoluble fractions of Capan-1 cells. These results suggested that type II PKA localized to a specific insoluble compartment stimulated CFTR-mediated current.

DISCUSSION

cAMP analogs selective for the A or B site of a specific regulatory subunit of PKA can be paired to synergistically activate a specific type of PKA, and a specific outcome of PKA activation may be measured. This approach has been used to determine the role of PKA types in a variety of systems. The use of type-selective analog pairs has implicated type I PKA activity in the
inhibition of human T lymphocyte proliferation (26), in leutinizing hormone-stimulated steroidogenesis in rat Leydig cells (21), and in the control of corticosterone production by zona fasciculata/reticularis cells (30), whereas the activity of type II PKA has been shown to be predominantly responsible for human placental renin secretion associated with β-adrenoceptor activation (9), growth inhibition of HL-60 human promyelocytic leukemia cells (24), and aldosterone production by zona glomerulosa cells (30). In our studies we applied type-selective pairs of analogs to Capan-1, T84, and Calu-3 cells and measured the effect of these analogs on CFTR-mediated chloride secretion. Although it was well documented that PKA phosphorylates CFTR, the specific type of PKA involved in various cell types had not been determined.

Application of the type II-selective analog pair CPT-cAMP plus N6-benzoyl-cAMP resulted in stimulation of chloride secretion as measured by isotopic flux experiments (Fig. 1, Table 2) in Capan-1, T84, and Calu-3 cells. The sustained nature of the efflux stimulated, the calcium independence of the efflux, the sensitivity of the current to specific chloride channel blockers, and the localization of the effect to a channel in the apical membrane suggested that the chloride channel stimulated by the type II-selective analogs was CFTR (Fig. 2, Tables 3 and 4). Although type II-selective analogs stimulated a larger change in $I_{sc}$ in all three cell types, type I-selective analogs did have some effect on chloride secretion in Capan-1 and Calu-3 cells. Current was activated in Capan-1 cells with the type I-selective analogs, and this current was at most partially blocked by DIDS and fully blocked by glibenclamide. These analogs may be stimulating CFTR by activating some type II PKA or through a “spillover” effect of type I activating CFTR. Alternatively, another apical channel may be stimulated. Similarly, the type I-selective analogs activated a calcium-dependent chloride efflux in Calu-3 cells that was not apparent with $I_{sc}$.
measurements. In this case, the type I-selective analogs might bestimulating a calcium-dependent chloride channel through the mobilization of calcium by an increase in cAMP levels; the presence of this channel could be culture dependent, inasmuch as Calu-3 cells are generally nonpolarized in the efflux assays but are polarized on the permeable supports used for the Ussing chamber experiments. Our experiments suggested that type II PKA played the predominant role in CFTR-mediated chloride secretion in Capan-1, T84, and Calu-3 cells.

Type I- and type II-selective analog pairs were able to stimulate soluble PKA activity to similar levels in Capan-1 and T84 cells (Fig. 3). This result seemed confusing in light of the fact that the analog pairs had significantly different effects on chloride secretion. This paradox was further complicated by the fact that addition of forskolin after the type I-selective analogs significantly increased current, whereas it had little additional effect after type II-selective analogs (Fig. 4). This result suggested that the type II-selective analogs and forskolin acted through the same pathway, inasmuch as they were not additive, and that type I-selective analogs may overlap in effect but were not identical to forskolin. Measurement of insoluble PKA activity after incubation with the type-selective analog pairs provided an explanation for this observation. At the concentrations used, type II-selective analogs stimulated three times as much insoluble PKA activity as did the type I-selective analog pair (Fig. 5). This result suggested that the stimulation of a specifically localized PKA activity was important in CFTR-mediated chloride secretion.

Compartmentalization of PKA activity to enhance specificity and speed of phosphorylation of substrates is not a novel idea (7, 20). Specific proteins, AKAPs, have been isolated that bind to the RII subunit and may localize type II PKA to specific regions of the cell, such as to the cortical actin cytoskeleton by AKAP7 (18, 22) or to the sarcoplasmic reticulum by AKAP100 (19). An AKAP has also been isolated that not only binds type II PKA but also phosphatase 2B and, thereby, localizes phosphorylating and dephosphorylating activities close to the substrate to enhance response rates to stimuli (8). Although we have not colocalized type II PKA with CFTR in this study, it is interesting to speculate that the insoluble PKA activity stimulated by the type II-selective analogs is situated close to CFTR, and only activation of this compartmentalized PKA will phosphorylate CFTR.

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REFERENCES


22. Moger, W. H. Evidence for the compartmentalization of adenosine 3’,5’-monophosphate (cAMP)-dependent protein kinases in...


