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cAMP-REGULATED CHLORIDE CURRENTS IN CHO CELLS

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We examined whether elevations in cAMP levels increase membrane chloride permeability in native CHO cells by measuring whole cell chloride currents and efflux of ^{125}I and ^{36}Cl. With 20 μM forskolin, no significant effect was seen on whole cell currents. However, 100 μM forskolin increased both whole cell chloride currents and the rate of ^{125}I and ^{36}Cl efflux. Forskolin-activated currents showed a linear current-voltage relationship in solutions with symmetrical chloride concentrations and reversal potential changed in the direction anticipated for a chloride-selective current when chloride was replaced with gluconate. These results indicate that native CHO cells exhibit cAMP-regulated chloride conductance pathways which become apparent only after large elevations in intracellular cAMP levels.

Chinese hamster ovary (CHO) cells have been widely used as a model to study different components of cAMP-dependent intracellular signal transduction pathways. In other cell types, membrane ion channels serve as an important effector mechanism for cAMP-dependent signalling (1-4). Consequently, we have evaluated the effects of increases in intracellular cAMP on membrane permeability in native CHO cells. Our studies indicate that, with relatively large increases in cAMP levels, an increase in membrane chloride conductance pathways is revealed.

METHODS

Patch Clamp Recording: Patch clamp recording techniques (5) were used to record membrane currents in CHO cells cultured in Ham's F12 media. Macroscopic currents were measured in the whole cell configuration. Patch pipettes were pulled from Corning 7052 glass and had resistances of 3-6 megaohms when filled with intracellular buffer (see below). Recordings were made with an Axopatch-1D amplifier. Voltage commands and current measurements were performed using pCLAMP programs (Axon Instruments), a Tl-125 interface (Axon Instruments) and a Compaq 386

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computer. Currents were filtered at 1 kHz and sampled at 2 kHz. Outward membrane currents and membrane depolarization are shown as positive.
Isotope studies: ^{125}I and ^{36}Cl (Amersham Labs) were used as markers for chloride efflux in cells grown to 80-90% confluence. Cells were loaded with isotope by 60 minute incubation in extracellular buffer containing 5-10 μCi/ml of ^{125}I and/or 5 μCi/ml ^{36}Cl. Cells were then washed with isotope free buffer to remove extracellularly bound isotope. The rate of isotope efflux was monitored every 30 seconds by measuring timed samples in a gamma counter for ^{125}I studies or with a liquid scintillation counter for studies measuring both ^{125}I and ^{36}Cl efflux. The effects of elevating cAMP levels were determined by the addition of forskolin (Sigma Scientific Co.) to the extracellular buffer. At the conclusion of the experiment, cells were lysed with 0.1 N NaOH and counted to determine the amount of isotope remaining in the monolayer.
cAMP Assay: Intracellular cAMP was measured by a radioimmunoassay in intact cells as previously described (6).
Solutions and Analysis: The extracellular buffer contained (in mM): NaCl 140; KCl 4; KH_2PO_4 1; $MgCl_2$ 2; $CaCl_2$ 1; glucose 10; and HEPES 10 (pH 7.35 with NaOH). Unless otherwise specified, the pipette solution contained (in mM): KCl 140; $MgCl_2$ 2; NaCl 10; $CaCl_2$ 0.05; EGTA 1.0; and HEPES 10 (pH 7.35 with KOH). Values are given as mean ± SE. "n" refers to the number of cells for electrophysiologic studies or number of monolayers for efflux studies. Statistical comparisons were by Student's t-test with a significance level of $p < 0.05$.

RESULTS and DISCUSSION

cAMP Level Determination: Intracellular cAMP was measured in intact cells following exposure to increasing concentrations of forskolin. The threshold for forskolin-induced increases in cAMP was approximately 1 μM. Basal and maximally stimulated cAMP levels were 222 ± 15 pmol/well and 4230 ± 212 pmol/well, respectively. EC_{50} and maximal responses occurred at 20 and 300 μM. 100 μM forskolin was approximately an EC_{90} dose.
Forskolin-Stimulated Whole Cell Currents: After gaining access to the cell interior, membrane potential was held at -40 mV and current responses were measured during 20 mV (400 msec duration) voltage steps to test potentials between -120 and 100 mV. Basal currents in unstimulated CHO cells were generally less than 200 pA. As reported in other studies (1), addition of 20 μM forskolin had no significant effect on whole cell currents (n=5) (Table 1). However, higher concentrations of forskolin (100 μM) stimulated currents within 3 minutes. The increase seen at +80 mV was 415 ± 136% of basal values ($p < 0.05$) and occurred in 9 of 12 cells studied (Table 1). In solutions with equal concentrations of chloride in the pipette and bath, current-voltage relationships were

TABLE 1

EFFECTS OF FORSKOLIN ON ^{125}I EFFLUX AND MACROSCOPIC CURRENTS

TREATMENT	PERCENT OF BASAL
Forskolin (20 μM)	
^{125}I	120 ± 3* (n=4)
Current at +80 mV	134 ± 22 (n=5)
Forskolin (100 μM)	
^{125}I	207 ± 37* (n=18)
Current at +80 mV	415 ± 136* (n=9)

Following the addition of forskolin, the rate of ^{125}I efflux and the peak currents at +80 mV are expressed as a percentage of basal values. * indicates $p < 0.05$

linear and the measured current reversal potential of -10.2 ± 3.6 mV (n=19) was near the theoretical reversal potential for chloride ions (0 mV). The chloride dependence of this current was examined by partial substitution of potassium chloride in the pipette solution with potassium gluconate. This maneuver produced a shift in the reversal potential to -28.0 ± 3.0 mV (n=5) (figure 1). This shift in reversal potential is in the direction anticipated for a chloride selective current. The outward

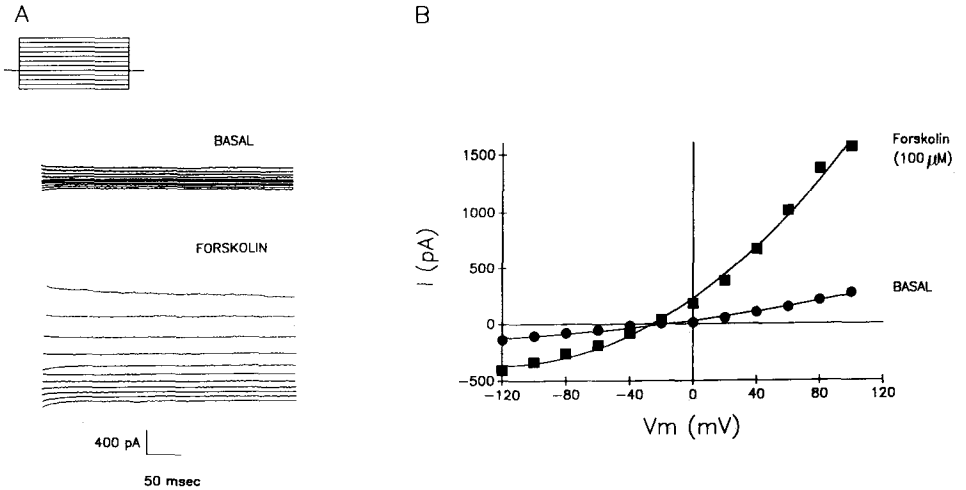

Figure 1: Stimulation of whole cell chloride currents by 100 μM forskolin. (A) An increase in whole cell currents was noted within 3 minutes of the addition of 100 μM forskolin to the extracellular solution. The inset shows the voltage protocol used to measure current-voltage relationships. From a holding potential of -40 mV, 20 mV (400 msec duration) voltage steps between -120 and 100 mV were performed. (B) The corresponding current-voltage relationship is shown. In this example, pipette potassium chloride was partially substituted with potassium gluconate and whole cell reversal potential was near E_{Cl} (-44 mV).

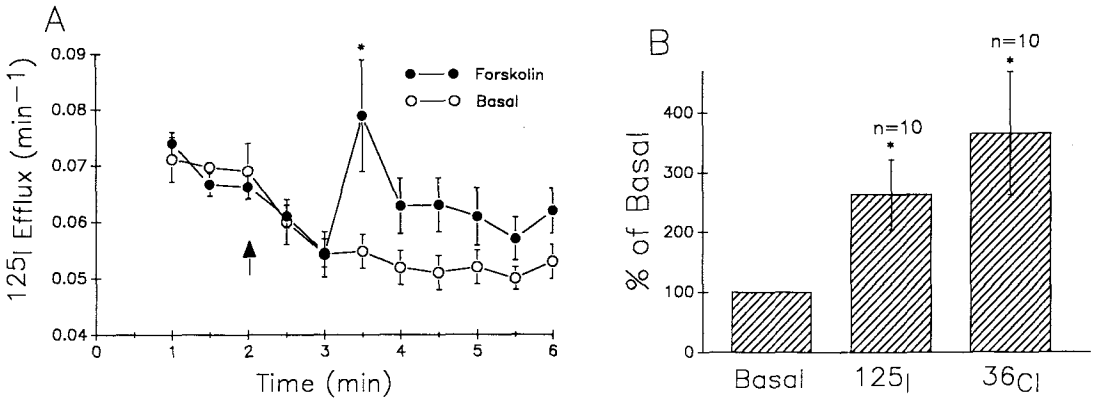

Figure 2: Forskolin-induced stimulation of ^{125}I and ^{36}Cl efflux in CHO cells. (A) In this representative study, addition of 100 μM forskolin (at arrow; n=4) produced an increase in ^{125}I efflux as compared to the basal rate of efflux (n=4). (B) In cells loaded with both ^{125}I and ^{36}Cl, exposure to forskolin caused a simultaneous increase in efflux of both isotopes (n=10). The relative stimulation with respect to the basal levels was greater for ^{36}Cl than ^{125}I (* $p < 0.05$).

rectification shown in figure 1 reflects the asymmetric chloride concentrations in the pipette and bath solutions.

Efflux Studies: ^{125}I efflux measurements have been utilized as a marker for membrane chloride permeability (7). A small, but significant, increase in efflux was seen with 20 μM forskolin (Table 1). However, with 100 μM forskolin a greater stimulation of ^{125}I efflux to 207 ± 37% (n = 18; $p < 0.05$) of the basal rate occurred. To confirm that ^{125}I is an appropriate marker for chloride permeability, additional studies were performed in cells loaded with both ^{125}I and ^{36}Cl and the results are summarized in figure 2B. Exposure to forskolin caused a simultaneous increase in efflux of both isotopes (n = 10). When compared to basal efflux rates, the response was larger for ^{36}Cl than ^{125}I, indicating that the forskolin-activated efflux pathway may have greater permeability for chloride than iodide.

Forskolin-stimulated increases in macroscopic chloride currents and efflux of ^{125}I and ^{36}Cl indicate that native CHO cells exhibit a cAMP-dependent chloride conductance pathway. Relatively high elevations in cAMP levels were required for current activation. This observation is consistent with previous reports (1) where 20 μM forskolin had no effect

on whole cell currents in native CHO cells. Interestingly, transfection of CHO cells with the cystic fibrosis transmembrane conductance regulator (CFTR) results in a large increase in chloride currents after smaller elevations in cAMP (1,2). This reflects expression of novel cAMP-dependent chloride channels after transfection (3,4). Our study suggests that a class of endogenous, cAMP-dependent, chloride channels are also present and after larger increases in cAMP levels become apparent.

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