Chloride Channels in Cystic Fibrosis Patients

Two recent reports (*1, 2*) concerning regulation of epithelial chloride channels by protein kinase C (PKC) appear to find opposite effects of phosphorylation by PKC at Ca^{2+} levels of approximately 1 μM. Hwang *et al.* (*1*) report activation of channels by PKC at a calculated free Ca^{2+} level of 1.2 μM, while Li *et al.* (*2*) report *inactivation* at free Ca^{2+} levels of 1 μM. As shown by Li *et al.*, the effect of PKC is sensitive to the concentration of free Ca^{2+}. Given that, the apparent discrepancy may relate, at least in part, to the way the concentrations were estimated by the two groups. Hwang *et al.* estimated that 0.5 mM $CaCl_2$, 1 mM EGTA, and 2 mM Mg^{2+} at *p*H 7.3 gives a free Ca^{2+} concentration of 1.2 μM, while Li *et al.* estimate that the same formulation gives a free Ca^{2+} concentration of 150 nM. We estimate that this formulation should yield a free Ca^{2+} concentration of approximately 110 nM (*3*), a level not considered unusual for resting cells. Thus, the results of the two groups are not as different as they initially appear. Using the solution formulation outlined above, Li *et al.* reported both activation and inactivation of the chloride channel, while Hwang *et al.* reported activation. When Li *et al.* increased free Ca^{2+} level to 720 nM [0.87 mM added Ca^{2+} and remaining formulation as above, free Ca^{2+} estimated by our calculation], inactivation clearly predominated.

Although the physiological significance of channel regulation by PKC remains to be determined in these cells, the above considerations suggest that either inhibition or excitation might be possible within physiological ranges of cytosolic free Ca^{2+}. Both groups agree that PKC activation of epithelial chloride channels is defective in the human genetic disease cystic fibrosis.

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REFERENCES AND NOTES

1. T.-C. Hwang *et al.*, *Science* **244**, 1351 (1989).
2. M. Li *et al.*, *ibid.*, p. 1353.
3. In our calculation, we assumed that the EGTA used in the experiments was 100% pure, and we used the metal ion–EGTA association constants from appendix A of J. R. Blinks *et al.* [*Prog. Biophys. Molec. Biol.* **40**, 1 (1982)]. These constants, which Blinks *et al.* termed "mixed association constants," were corrected for the fact that *p*H is a measure of hydrogen ion activity rather than concentration. We accounted for the binding of Mg^{2+} to EGTA using the method outlined by H. Portzehl *et al.* [*Biochem. Biophys. Acta* **79**, 581 (1964)].

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Response: At the time that our paper was published, we were not aware of the possibility that the magnitude of intracellular free Ca^{2+} could influence whether epithelial Cl^- channels are activated or inactivated by protein kinase C (PKC). Our original free Ca^{2+} estimates were made with a Ca^{2+} macroelectrode in the presence of 2 mM Mg^{2+}. Since the Ca^{2+} macroelectrode is sensitive to Mg^{2+} (selectivity ratio of 80:1, Ca^{2+}:Mg^{2+}), the high Mg^{2+} concentration in our solutions resulted in an overestimate of the free Ca^{2+} concentration. Since then we have remeasured the free Ca^{2+} concentration in our solutions with the Ca^{2+}-sensitive dye fura-2, using the same solution reported in our paper (0.5 mM $CaCl_2$, 1 mM EGTA, and 2 mM Mg^{2+}). The free Ca^{2+} measured with fura-2 is 116 nM. Thus, PKC can activate epithelial Cl^- channels at physiological levels of free Ca^{2+}. To address whether PKC activation can occur at higher Ca^{2+} concentrations, we had performed additional experiments that were not included in the original paper. We exposed Cl^- channels to PKC and adenosine triphosphate with 2 mM Mg^{2+} in the absence of any DAG but in the presence of high Ca^{2+} (70 μM, estimated with a Ca^{2+} macroelectrode without the solution Mg^{2+}). In this condition, we noted activation of Cl^- channels (3/3). Thus, in this condition PKC can activate Cl^- channels at high Ca^{2+} concentrations. Our conclusion remains that PKC activates epithelial Cl^- channels.

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Response: Because of the comments of Wine and Solc, we measured the free [Ca^{2+}] in our solution using fura-2 and the procedures and calibration methods described by Grynkiewicz *et al.* (*1*). The solution we reported (*2*) with 150 nM free Ca^{2+} had 136 nM Ca^{2+}, the low Ca^{2+} (<10 nM) solution had unmeasurable Ca^{2+}, and the high (1 μM) [Ca^{2+}] solution had 688 nM Ca^{2+} (but note that because of the shape of the fura-2/Ca^{2+} curve, this measurement is less reliable).

In cell-free patches of membrane, we examined the effect of protein kinase C (PKC) at a high [Ca^{2+}] (688 nM and greater), because biochemical studies have shown that PKC is maximally activated at high [Ca^{2+}] (*3*). Under those conditions we found that PKC inactivated the Cl^- channel. However at a low [Ca^{2+}] (<10 nM), PKC activated the channel. At 140 nM Ca^{2+}, we reported variable effects: either inactivation or activation in individual cases. These opposite effects on the Cl^- channel were paralleled by the results we obtained in intact cells: stimulation of PKC with phorbol ester could either activate or inactivate Cl^- channels. Thus our report and that of Hwang *et al.* (*4*) are in reasonable agreement at low to intermediate [Ca^{2+}].

Wine and Solc raise an interesting question about the physiologic significance of PKC in regulating the Cl^- channel. We have shown that bradykinin, which activates phospholipase C, and isoproterenol, which increases cellular cAMP (adenosine 3′,5′-monophosphate) levels, both increase the cellular mass of diacylglycerol, an activator of PKC (*5*). In addition we have shown that these two agonists transiently increase the cellular free [Ca^{2+}] (*6*). These observations suggest that PKC is activated in response to receptor-mediated stimuli, but it is difficult to predict the effect of PKC on the Cl^- channel in the intact cell. Although we favor the notion that PKC may function as a negative regulator of the Cl^- channel, thereby modulating the effect of PKA, further studies are required.

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