Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 963–967, February 1988
Physiological Sciences

Insulin and phorbol ester stimulate conductive Na^+ transport through a common pathway

(apical Na^+ permeability/frog skin/protein kinase C)

MORTIMER M. CIVAN*†, KIM PETERSON-YANTORNO*, AND THOMAS G. O'BRIEN‡

Departments of *Physiology and †Medicine, University of Pennsylvania School of Medicine, and the ‡Wistar Institute, Philadelphia, PA 19104

Communicated by Robert E. Forster, October 6, 1987 (received for review July 22, 1987)

ABSTRACT **Insulin stimulates Na^+ transport across frog skin, toad urinary bladder, and the distal renal nephron. This stimulation reflects an increase in apical membrane Na^+ permeability and a stimulation of the basolateral membrane Na,K-exchange pump. Considerable indirect evidence has suggested that the apical natriferic effect of insulin is mediated by activation of protein kinase C. However, no direct information has been available documenting that insulin and protein kinase C indeed share a common pathway in stimulating Na^+ transport across frog skin. In the present work, we have studied the interaction of insulin and phorbol 12-myristate 13-acetate (PMA), a documented activator of protein kinase C. Preincubation of skins with 1,2-dioctanoylglycerol, another activator of protein kinase C, increases baseline Na^+ transport and reduces the subsequent natriferic response to PMA. Preincubation with PMA markedly reduces the subsequent natriferic action of insulin. This effect does not appear to primarily reflect PMA-induced internalization of insulin receptors. The insulin receptors are localized on the basolateral surface of frog skin, but the application of PMA to this surface is much less effective than mucosal treatment in reducing the response to insulin. Preincubation with D-sphingosine, an inhibitor of protein kinase C, also reduces the natriferic action of insulin. The current results provide documentation that insulin and protein kinase C share a common pathway in stimulating Na^+ transport across frog skin. The data are consistent with the concept that the natriferic effect of insulin on frog skin is, at least in part, mediated by activation of protein kinase C.**

In addition to its well-characterized effects on carbohydrate, lipid, and protein metabolism, insulin exerts a number of actions on electrolyte transfer (1). Insulin plays a major role in regulating the distribution of K^+ between the extra- and intracellular fluids and also stimulates Na^+ transport across the distal nephron (2, 3), frog skin (4), and toad urinary bladder (5). These electrolyte effects are likely triggered by specific binding of hormone to insulin receptors at the basolateral membrane, since stimulation of Na^+ transport can be observed at concentrations at least as low as 0.5–0.7 nM (2, 6). The hormonal actions on electrolyte transport can be dissociated from those on glucose and amino acid transport (7–9).

Insulin exerts its natriferic effect on frog skin by at least two mechanisms, a direct stimulation of the Na,K-exchange pump (10, 11) and an increase in the apical Na^+ permeability (12, 13). The intracellular mediators are unknown. Much information now suggests that many of the transport-unrelated hormonal effects may be mediated by a small glycophospholipid (14, 15). Other effects of insulin may be mediated by the intracellular alkaline shift resulting from hormonal stimulation of Na/H exchange through a membrane antiport (16).

Considerable evidence suggests that the apical natriferic effect of insulin is mediated by activation of protein kinase C (17). However, this evidence has been entirely indirect. No information has been available to indicate whether or not insulin and protein kinase C activation stimulate Na^+ transport through a common pathway. In the present manuscript, we report observations of the interactions of insulin and protein kinase C activation and inhibition in stimulating Na^+ transport across frog skin.

MATERIALS AND METHODS

Tissue and Solutions. Abdominal skins were excised from doubly pithed frogs (Northern variety *Rana pipiens*, West Jersey Biological Supply, Wenonah, NJ). The skins were rinsed and bathed with Ringer's solution (18, 19) (120.0 mM Na^+/3.5 mM K^+/1.0 mM Ca^{2+}/118.0 mM Cl^-/2.5 mM HCO_3^-/10.0 mM Hepes, osmolality 240 mosmol/kg, pH 7.6).

The tissues were mounted between the two halves of a Lucite double chamber. Simultaneous control and experimental measurements were performed on the same tissue by studying two adjoining (0.79 cm^2) areas of skin. The transepithelial electrical potential [serosal (inner) with respect to mucosal (outer) surface] was voltage clamped to 0 mV except for 5-sec intervals during which the transepithelial electrical potential was clamped at 10 mV. The transepithelial current was continuously recorded with a dual-pen recorder. The value of transepithelial current necessary to clamp transepithelial electrical potential at 0 mV is termed the short circuit current (I_{SC}) and is equal to the net transport of Na^+ across this tissue (20). The transepithelial slope conductance was measured as the ratio of the deflection of the transepithelial current to the 10-mV step in voltage.

Chemicals. Phorbol 12-myristate 13-acetate (PMA) was purchased from Chemicals for Cancer Research (Eden Prairie, MN), and 1,2-dioctanoylglycerol [$(Oco)_2Gro$] was from Avanti Polar Lipids. The $(Oco)_2Gro$ was prepared by evaporating the chloroform solvent from the preparation received from the supplier and redissolving the diacylglycerol in dimethyl sulfoxide. PMA and D-sphingosine (Sigma) were dissolved in ethanol. Porcine insulin, donated by Eli Lilly in crystalline form (lot 615-2H2-300; 26.8 units/mg), was dissolved in 0.005 M HCl. Vasopressin was purchased as [8-arginine]vasopressin (Calbiochem–Behringer) and dissolved in deionized water. The amiloride was kindly provided by George M. Fanelli, Jr. (Merck Institute for Therapeutic Research, West Point, PA).

Data Presentation. Unless otherwise stated, the data are presented as means ± SEM. The probability (P) of the null hypothesis has been calculated using Student's t test.

RESULTS

Interaction of PMA and $(Oco)_2Gro$. Fig. 1*A* presents the transepithelial currents monitored across the experimental

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: PMA, phorbol 12-myristate 13-acetate; $(Oco)_2Gro$, 1,2-dioctanoylglycerol; I_{SC}, short circuit current.

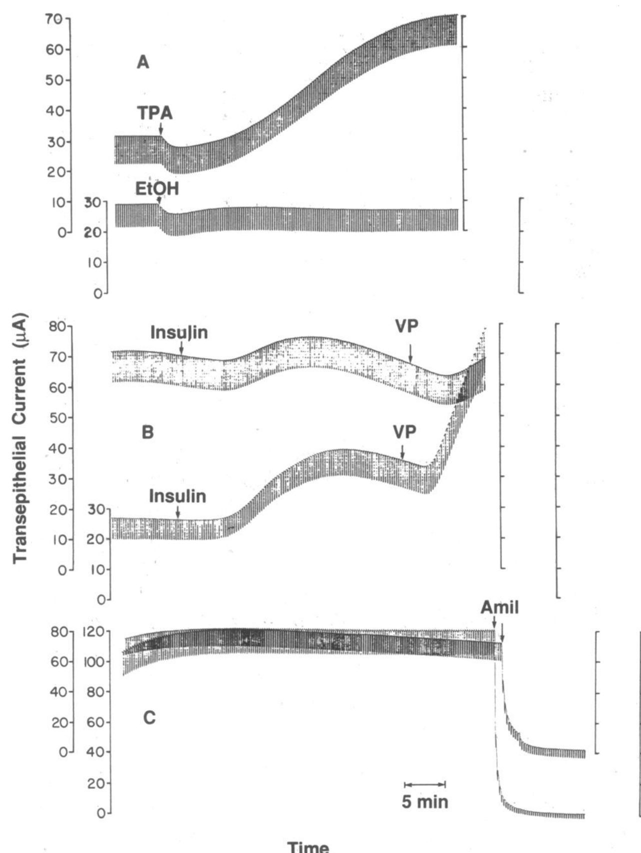

FIG. 1. Effect of PMA (TPA) on subsequent natriferic response to insulin. Consecutive recordings from a single frog skin are shown. The upper envelope of each trace is the I_{SC}. The lower envelope is the transepithelial current required to clamp the transepithelial potential to 10 mV (serosa positive with respect to mucosa). Thus, the displacement of the two leaves is proportional to the transepithelial conductance. (*A*) After a stable baseline state was attained, the experimental area was treated with 160 nM PMA on both the mucosal and serosal surfaces, while the control area of the same skin was exposed to an equal volume of ethanol solvent. The control trace displayed only a transient response, in contrast to the very large, sustained increase in I_{SC} across the experimental tissue. (*B*) After the peak response to PMA, 3 μM insulin was added to both serosal reservoirs. The hormone stimulated the I_{SC} of the control (PMA-untreated area) by 76%, while increasing I_{SC} across the PMA-pretreated tissue by only ≈13%. The limited ability of the PMA-pretreated area to respond to insulin did not reflect saturation of the Na^+ transport system, since the subsequent addition of vasopressin (VP) (100 milliunits/ml) still stimulated I_{SC}, albeit to a lesser degree than across the control tissue area. (*C*) At the conclusion of the experiment, addition of 100 μM amiloride (Amil) blocked Na^+ transport, abolishing the I_{SC}, and reducing the transepithelial conductance across both the experimental and control areas of skin.

area (upper trace) and control area (lower trace) of a single skin. The upper envelope of each trace is the I_{SC}; the displacement of the two leaves of each trace is proportional to the transepithelial conductance. The addition of 160 nM PMA to the mucosal and serosal reservoirs bathing the experimental area produced a doubling of I_{SC}, whereas addition of an equal volume of ethanol solvent produced only a small, transient response. Although a supramaximal concentration was used in this experiment, the K_m for stimulating Na^+ transport across frog skin is ≈3 nM (19). This stimulation of I_{SC} is likely to arise from protein kinase C activation since the effect can be replicated by other activators of protein kinase C [$(Oco)_2$Gro and 1-oleoyl-2-acetylglycerol] (17).

In quantifying the results of records such as those of Fig. 1, we have measured the increment in I_{SC} as the peak response minus the baseline value. When the current was unchanging at the time of the experimental perturbation (Fig. 1*A*), the baseline was simply taken to be the I_{SC} just before the experimental change. When the current was falling (Fig. 1*B*), the baseline value was estimated by extrapolating I_{SC} from its rate of decline (just prior to the perturbation) to the time at the peak effect. Where expressed as percentages, the experimental changes have been normalized to the I_{SC}s measured at the beginning of the experiment.

If the PMA-induced stimulation of I_{SC} reflects activation of protein kinase C, we would expect a negative interaction between supramaximal doses of PMA and $(Oco)_2$Gro. Diacylglycerols are considered to be physiologic activators of protein kinase C (21). This prediction was tested in a series of five skins. The I_{SC}s across the areas treated with 375 μM $(Oco)_2$Gro increased by 9 ± 3 μA/cm² (31 ± 9%) and were unchanged [0.6 ± 0.4 μA/cm² (2 ± 1%)] across the control tissue areas. Averaging the paired differences between experimental and control areas, $(Oco)_2$Gro increased the I_{SC} by 8 ± 3 μA/cm² (29 ± 10%). The pretreatment with diacylglycerol also inhibited the subsequent response to supramaximal concentrations of PMA. The I_{SC} of the control (untreated) areas increased by 27 ± 12 μA/cm² (79 ± 28%), whereas that of experimental [$(Oco)_2$Gro-pretreated] areas rose by 18 ± 9 μA/cm² (53 ± 14%). The paired difference was −9 ± 5 μA/cm² (−26 ± 16%). The large SEM reflected the broad range in tissue responsiveness to PMA in this series of tissues, increasing by 7–63 μA/cm² (36–187%). However, in each case, the prior exposure to $(Oco)_2$Gro reduced the percentage increase in current subsequently elicited by PMA; therefore, even by the nonparametric coin test, the probability (*P*) of the null hypothesis was $(0.5)^5 < 0.05$. Thus, activation of protein kinase C by applying $(Oco)_2$Gro to frog skin reduced the subsequent natriferic response to PMA by ≈30%. This observation confirms the expectation that the sequential addition of supramaximal concentrations of two different activators of protein kinase C results in a less than additive stimulation of Na^+ transport across frog skin.

Interaction of Insulin and PMA. In a series of nine experiments, 160 nM PMA was introduced into the mucosal and serosal solutions bathing the experimental area, increasing

Table 1. Effect of PMA pretreatment on natriferic response to insulin

Tissue area	Baseline I_{SC}, μA/cm²	Change in I_{SC} after addition, μA/cm²: PMA	Insulin to exp. and con.	VP to exp. and con.
Experimental	28 ± 4	21 ± 4	3 ± 2	13 ± 5
Control	27 ± 4	0.1 ± 0.3	9 ± 3	49 ± 11
Difference	0 ± 2	21 ± 4 ($P < 0.005$)	−6 ± 2 ($P < 0.02$)	−36 ± 7 ($P < 0.005$)

The experimental area of each of nine skins was exposed to 160 nM mucosal and serosal PMA. Thereafter, both experimental (exp.) and control (con.) areas were sequentially treated with 3 μM serosal insulin and vasopressin (VP) at 100 milliunits/ml. At the conclusion of each experiment, 100 μM amiloride was added to each mucosal surface to abolish the I_{SC}, verifying that the I_{SC} continued to reflect net Na^+ transport across the tissue.

Table 2. Sidedness of hormonal inhibition by PMA

Tissue area	Baseline I_{SC}, $\mu A/cm^2$	Change in I_{SC} after addition, $\mu A/cm^2$ PMA	Insulin	VP
Mucosal PMA	37 ± 11	21 ± 4	1.3 ± 0.5	12 ± 4
Serosal PMA	37 ± 9	7 ± 3	9 ± 2	45 ± 8
Difference	0 ± 3	14 ± 4 ($P < 0.02$)	−8 ± 2 ($P < 0.05$)	−33 ± 6 ($P < 0.01$)

Adjoining areas of the same skin were exposed to 160 nM PMA. One tissue area was treated with mucosal PMA, while the adjoining area was exposed to serosal PMA. To the reservoirs not receiving PMA, an equal volume of ethanol solvent was added. As in the case of the experiments in Table 1, thereafter, both tissue areas were sequentially treated with 3 μM serosal insulin and vasopressin (VP) at 100 milliunits/ml.

I_{SC} by 21 ± 4 $\mu A/cm^2$ (95 ± 21%) (Table 1 and Fig. 1). Addition of ethanol solvent to the control area produced a negligible response. The paired difference was 21 ± 4 $\mu A/cm^2$ (93 ± 20%).

The pretreatment with PMA had a dramatic effect on the subsequent responsiveness of the tissue to insulin added at the time of the peak PMA action. The I_{SC} continued to decline after hormonal addition, displaying little response to insulin. The hormonal effect was also found to be reduced if addition of insulin was delayed until the current reached a new, steady baseline value (unpublished data). In the present study, insulin increased the I_{SC} of the control (PMA-untreated area) by 9 ± 3 $\mu A/cm^2$ (33 ± 10%), and by only 3 ± 2 $\mu A/cm^2$ (12 ± 5%) after exposure to PMA. The paired difference in the response of net Na^+ transport to insulin was −6 ± 2 $\mu A/cm^2$ (−21 ± 7%). Thus, prior exposure to PMA reduced the natriferic response to insulin by approximately two-thirds.

Vasopressin (100 milliunits/ml) was subsequently added at the time of peak response to insulin, or shortly thereafter. As reported (19, 22), vasopressin was much more effective in stimulating Na^+ transport across the tissue area not previously exposed to PMA (Fig. 1 and Table 1). However, the hormone did increase I_{SC} across each of the nine experimental tissue areas pretreated with PMA. The mean increment was 13 ± 5 $\mu A/cm^2$ (55 ± 16%) following the sequential addition of PMA and insulin to the experimental area.

Thus, the blunted response of the PMA-pretreated areas to insulin did not reflect saturation of the Na^+ transporting system, since the subsequent addition of vasopressin did stimulate I_{SC} across the same tissues.

Sidedness of the Inhibition of Hormonal Action by PMA. The results of Fig. 1 and Table 1 support the concept of a negative interaction between insulin and protein kinase C activation in stimulating Na^+ transport across frog skin. However, PMA could possibly have inhibited the hormonal response by internalizing or down-regulating the insulin receptors. We have examined this possibility by examining the sidedness of the effect of PMA. In a series of five experiments, 160 nM PMA was added to the mucosal medium bathing one tissue area, while the same concentration of PMA was applied to the serosal surface of the adjoining area. An identical amount of ethanol solvent was added to the other two reservoirs. The mucosal-PMA stimulated I_{SC} by 21 ± 4 $\mu A/cm^2$ (67 ± 12%). The serosal PMA had far-less effect (Table 2 and Fig. 2); I_{SC} increased by 7 ± 3 $\mu A/cm^2$ (18 ± 7%). By paired analysis, the difference in stimulation elicited by PMA from the two surfaces was 14 ± 4 $\mu A/cm^2$ (49 ± 13%). In each experiment, prior exposure to PMA was also far more effective on the mucosal than on the serosal surface in inhibiting the subsequent natriferic response to 3 μM insulin. The insulin was added only to the serosal medium, since it is ineffective from the contralateral side. The hormone increased I_{SC} by 9 ± 2 $\mu A/cm^2$ (24 ± 6%) following exposure to serosal PMA and by only 1.3 ± 0.5 $\mu A/cm^2$ (4 ± 2%) after mucosal PMA. By paired analysis, the difference in the response of I_{SC} to insulin was −8 ± 2 $\mu A/cm^2$ (−21 ± 6%) (Table 2). Thus, PMA was far less effective in inhibiting the action of insulin when added to the same tissue surface containing the insulin receptors than when added to the mucosal medium.

Effect of Protein Kinase C Inhibition on Response to Insulin. An ideally selective inhibitor of protein kinase C activity has not yet been identified. However, both sphingosine and the lysosphingosines have been reported to inhibit protein kinase C significantly in other tissues (23). In a series of six skins, the experimental area was initially exposed to 100 μM D-sphingosine, while the adjoining control area was exposed to identical volumes of ethanol solvent. Both areas were subsequently and sequentially treated with 33–98 nM insulin and vasopressin at 100 milliunits/ml. In each experiment, preincubation with D-sphingosine reduced the later natriferic effect of insulin (Table 3). The sphingosine also reduced the response to vasopressin (Table 3), suggesting that the inhibitor decreases cAMP-dependent kinase activity as well.

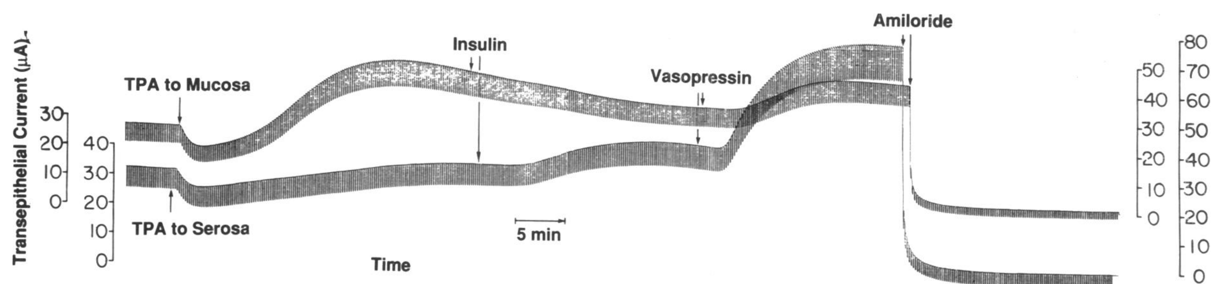

FIG. 2. Sidedness of the effect of PMA (TPA) on the tissue's subsequent natriferic response to insulin. PMA (160 nM) was first added to both the mucosal medium bathing the "experimental" area of skin and the serosal medium bathing the adjoining ("control") area; identical volumes of ethanol solvent were simultaneously added to the experimental serosal and control mucosal solutions. Following an initial transient decline, the I_{SC} of the control area (exposed to serosal PMA) increased only slightly, by ≈8%. In contrast, following exposure to mucosal PMA, the I_{SC} of the experimental skin area markedly increased by 85%. The subsequent addition of 3 μM insulin to both serosal reservoirs produced a significant increase in I_{SC} only across the tissue exposed to serosal PMA. As reported (19, 22), mucosal PMA was also far more effective than serosal PMA in blunting the subsequent natriferic response to vasopressin. Blocking apical Na^+ entry with 100 μM mucosal amiloride abolished the I_{SC}, confirming that the I_{SC} was reflecting net Na^+ transport across both tissue areas.

Table 3. Effect of sphingosine pretreatment on natriferic response to insulin

Tissue area	Baseline I_{SC}, $\mu A/cm^2$	Change in I_{SC} after addition, $\mu A/cm^2$		
		Sphingosine to exp.	Insulin to exp. and con.	VP to exp. and con.
Experimental	65 ± 10	−9 ± 4	9 ± 3	45 ± 11
Control	68 ± 11	−7 ± 2	21 ± 5	64 ± 13
Difference	−2 ± 4	−3 ± 5	−11 ± 4 ($P < 0.05$)	−19 ± 5 ($P < 0.02$)

Sphingosine (100 μM) was added to the mucosol and serosal media bathing each of six skins, while equal volumes of ethanol solvent were added to the solutions bathing the adjoining control surfaces. Thereafter, both experimental (exp.) and control (con.) areas were consecutively treated with serosal insulin and vasopressin (VP) at 100 milliunits/ml. In five of the six experiments, the insulin concentration was 33 nM; in the sixth, 98 nM insulin was applied.

DISCUSSION

A number of lines of indirect evidence have led us to suggest that protein kinase C is one mediator of insulin's stimulatory action of Na^+ transport across frog skin (17). (*i*) Insulin (4) and activators of protein kinase C (17, 19, 22) stimulate Na^+ transport across this tissue. (*ii*) In part, the natriferic action of insulin (12, 13) and protein kinase C activation (17) is expressed by increasing apical Na^+ permeability. (*iii*) In other cells, insulin increases diacylglycerol production by stimulating phospholipase C hydrolysis of a phosphatidylinositol glycan (24, 25) and by activating *de novo* phosphatidic acid synthesis (26). (*iv*) Diacylglycerol can be translocated from one plasma membrane to intracellular membranes (27) and presumably to the contralateral plasma membrane. (*v*) Diacylglycerol is thought to be a physiological trigger of protein kinase C activity in other cells (28). (*vi*) Insulin has been reported to increase protein kinase C activity in BC3H-1 myocytes (29).

Despite this body of indirect evidence, no direct information has been available to indicate whether insulin and protein kinase C activation share a common pathway in stimulating Na^+ transport across frog skin. This question has been addressed in the present study. The effects of sequentially adding $(Oco)_2Gro$ and PMA demonstrated that preincubation of the tissue with one activator [$(Oco)_2Gro$] of protein kinase C reduced the ability of a second activator (PMA) to stimulate transepithelial Na^+ transport. This documentation provided a rational basis for using transepithelial Na^+ transport as a probe for examining the interaction between one known protein kinase C activator (PMA) and a putative activator (insulin) of protein kinase C in frog skin. The results indicate that the natriferic response of the hormone is markedly inhibited by preincubation with PMA (Table 1 and Fig. 1).

In principle, PMA could have reduced the hormonal action by decreasing binding of insulin to its basolateral receptors. This possibility was examined in the present work by studying the sidedness of the effect of PMA. We reasoned that if PMA blocked insulin by either internalizing or down-regulating the hormonal receptors, its inhibitory effect should have been greater on the tissue surface containing the receptors. In fact, the PMA was far more effective in blocking the natriferic response of insulin when added to the contralateral mucosal surface (Table 2 and Fig. 2).

The present data document that insulin and PMA share one or more common steps in stimulating Na^+ transport across frog skin. Considered in isolation, this observation could be consistent with a parallel, independent stimulation of a single rate-limiting step by insulin and protein kinase C. However, as summarized above, a causal relationship between changes in external insulin concentration and intracellular protein kinase C has been reported in another tissue (29). This concept is further strengthened by our observation that preincubation of the protein kinase C inhibitor sphingosine reduces the natriferic action of insulin. We suggest that the most direct interpretation of the published data and current results is that insulin triggers a series of linear events leading to an enhancement of apical Na^+ permeability and that activation of protein kinase C is one critical step in that cascade of events.

In contrast to the marked stimulation of Na^+ transport by protein kinase C activation in intact frog skin, protein kinase C activation of the A6 cell line inhibits amiloride-sensitive I_{SC} (30). A range of intermediate effects has been noted in preliminary studies of other cells (31–33). This observation is not surprising since the phorbol esters have long been known to exert very different effects on different cells, even in terms of a single parameter (ref. 34, p. 99). The nature and magnitude of a given effect depend on the cell type, tissue, and species of origin, and state of differentiation. This phenomenon may reflect several different mechanisms. (*i*) Cells in culture can dedifferentiate, losing one or more regulatory molecules or pathways modulating Na^+ transport. (*ii*) More generally, subtle differences in the phospholipid microenvironment near the channel and access to the serine or threonine residues of the putative regulatory site may differ in different cells. (*iii*) Perhaps most importantly, it is now clear that there are several different forms of protein kinase C (35–38) and these may have different substrate specificities. The extent to which the various forms of protein kinase C mediate different effects of insulin in different tissues remains to be determined.

This work was supported in part by Grants AM-20632 and CA-36353 from the National Institutes of Health, and by The Research Foundation, The Medical School Biomedical Research Support Grant (BRSG) and The University BRSG of the University of Pennsylvania.

1. DeFronzo, R. A. & Bia, M. (1985) in *The Kidney: Physiology and Pathophysiology*, eds. Seldin, D. W. & Giebisch, G. (Raven, New York), pp. 1179–1206.
2. DeFronzo, R. A., Cooke, C. R., Andres, R., Faloona, G. R. & Davis, P. J. (1975) *J. Clin. Invest.* **55**, 845–855.
3. DeFronzo, R. A., Goldberg, M. & Agus, Z. S. (1976) *J. Clin. Invest.* **58**, 83–90.
4. Herrerra, F., Whittenbury, G. & Planchart, A. (1963) *Biochim. Biophys. Acta* **66**, 170–172.
5. Herrerra, F. C. (1965) *Am. J. Physiol.* **209**, 819–824.
6. Cobb, M. H., Yang, C.-P. H., Brown, J. A., Jr., & Scott, W. N. (1986) *Biochim. Biophys. Acta* **856**, 123–129.
7. Gelehrter, T. D., Shreve, P. D. & Dilworth, V. M. (1984) *Diabetes* **33**, 428–434.
8. Zierler, K. L. (1959) *Am. J. Physiol.* **197**, 524–526.
9. Zierler, K. L. (1960) *Am. J. Physiol.* **198**, 1066–1070.
10. Hougen, T. J., Hopkins, B. E. & Smith, T. W. (1978) *Am. J. Physiol.* **234**, C59–C63.
11. Siegel, B. & Civan, M. M. (1976) *Am. J. Physiol.* **230**, 1603–1608.
12. Erlij, D. & Schoen, H. F. (1981) *Ann. N.Y. Acad. Sci.* **372**, 272–288.
13. Schoen, H. F. & Erlij, D. (1985) *Pflügers Arch.* **405**, Suppl. 1, S33–S38.

14. Jarett, L., Wong, E. H. A., Macaulay, S. L. & Smith, J. A. (1985) *Science* **227**, 533–535.
15. Mato, J. M., Kelly, K. L., Abler, A. & Jarett, L. (1987) *J. Biol. Chem.* **262**, 2131–2137.
16. Moore, R. D. (1983) *Biochim. Biophys. Acta* **737**, 1–49.
17. Civan, M. M., Peterson-Yantorno, K. & O'Brien, T. G. (1987) *J. Membr. Biol.* **97**, 193–204.
18. Civan, M. M., Lin, L.-E., Peterson-Yantorno, K., Taylor, J. & Deutsch, C. (1984) *Am. J. Physiol.* **247**, C506–C510.
19. Mauro, T., O'Brien, T. G. & Civan, M. M. (1987) *Am. J. Physiol.* **252**, C173–C178.
20. Ussing, H. H. (1960) *Handb. Exp. Pharmakol.* **13**, 1–195.
21. Kaibuchi, K., Takai, Y. & Nishizuka, Y. (1981) *J. Biol. Chem.* **256**, 7146–7149.
22. Civan, M. M., Rubenstein, D., Mauro, T. & O'Brien, T. G. (1985) *Am. J. Physiol.* **248**, C457–C465.
23. Hannun, Y. A. & Bell, R. M. (1987) *Science* **235**, 670–674.
24. Fox, J. A., Soliz, N. M. & Saltiel, A. R. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 2663–2667.
25. Saltiel, A. R., Fox, J. A., Sherline, P. & Cuatrecasas, P. (1986) *Science* **233**, 967–972.
26. Farese, R. V., Konda, T. S., Davis, J. S., Standaert, M. L., Pollet, R. J. & Cooper, D. R. (1987) *Science* **236**, 586–589.
27. Pagano, R. E. & Longmuir, K. J. (1985) *J. Biol. Chem.* **260**, 1909–1916.
28. Castagna, M., Takai, Y., Kaibuchi, K., Sano, K., Kikkawa, U. & Nishizuka, Y. (1982) *J. Biol. Chem.* **257**, 7847–7851.
29. Cooper, D. R., Konda, T. S., Standaert, M. L., Davis, J. S., Pollet, R. J. & Farese, R. V. (1987) *J. Biol. Chem.* **262**, 3633–3639.
30. Yanase, M. & Handler, J. S. (1986) *Am. J. Physiol.* **250**, C517–C522.
31. Palmer, L. G. & Frindt, G. (1987) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **46**, 495 (abstr.).
32. Rorsman, P., Arkhammar, P. & Berggren, P.-O. (1986) *Am. J. Physiol.* **251**, C912–C919.
33. Schlondorff, D. & Levine, S. D. (1986) *J. Clin. Invest.* **76**, 1071–1078.
34. Diamond, L. (1984) *Pharmacol. Ther.* **26**, 89–145.
35. Brandt, S. J., Niedel, J. E., Bell, R. M. & Young, W. S., III (1987) *Cell* **49**, 57–63.
36. Coussens, L., Parker, P. J., Rhee, L., Yang-Feng, T. L., Chen, E., Waterfield, M. D., Francke, U. & Ullrich, A. (1986) *Science* **233**, 859–866.
37. Knopf, J. L., Lee, M.-H., Sultzman, L. A., Kriz, R. W., Loomis, C. R., Hewick, R. M. & Bell, R. M. (1986) *Cell* **46**, 491–502.
38. Parker, P. J., Coussens, L., Totty, N., Rhee, L., Young, S., Chen, E., Stabel, S., Waterfield, M. D. & Ullrich, A. (1986) *Science* **233**, 853–859.