Kidney International, Vol. 51 (1997), pp. 1037–1041

Tyrosine kinase, phosphatidylinositol 3-kinase, and protein kinase C regulate insulin-stimulated NaCl absorption in the thick ascending limb

OSAMU ITO, YOSHIAKI KONDO, MINAKO OBA, NOBUYUKI TAKAHASHI, KEN OMATA, and KEISHI ABE

Second Department of Internal Medicine and Department of Pediatrics, Tohoku University School of Medicine, Sendai, Japan

Tyrosine kinase, phosphatidylinositol 3-kinase, and protein kinase C regulate insulin-stimulated NaCl absorption in the thick ascending limb. We have previously shown a direct stimulatory effect of insulin on NaCl absorption in the medullary thick ascending limb of Henle's loop (mTAL). To further investigate the signal transduction involved, we determined whether tyrosine kinase, phosphatidylinositol 3-kinase (PI3-kinase), and/or protein kinase C (PKC) regulate insulin-stimulated NaCl absorption in the mTAL by *in vitro* microperfusion methods. In control experiments, insulin increased transepithelial voltage (V_{te}) and net lumen-to-bath Cl^- flux (J_{Cl}). Genistein and methyl 2,5-dihydroxycinnamate, two specific tyrosine kinase inhibitors, abolished the effects of insulin. Wortmannin, a specific PI3-kinase inhibitor, inhibited the action of insulin. The effects of insulin also were inhibited by staurosporin and calphostin C, which are dissimilar inhibitors of PKC. These results indicate that insulin stimulates NaCl absorption in the mTAL through tyrosine kinase, PI3-kinase, and PKC-mediated mechanisms. Moreover, because we have reported previously that insulin causes no detectable change in cytosolic free Ca^{2+} in the mTAL cells, the present results also suggest that insulin-induced PKC activation is not related to inositol 1,4,5-triphosphate (IP_3) production.

Insulin shows the antinatriuretic effect without changes in glomerular filtration rate or renal blood flow [1–4]. Clearance experiments [2, 3] and micropuncture studies [3, 4] have suggested that the antinatriuretic effect of insulin *in vivo* is localized mainly to the thick ascending limb of Henle's loop (TAL). Recently, we and another group provided the evidence by *in vitro* microperfusion methods that insulin directly stimulates NaCl absorption in the rabbit and mouse TAL [5, 6]. This effect of insulin in the TAL may be mediated by activation of NaCl absorption involving furosemide-inhibitable Na^+-K^+-$2Cl^-$ cotransport [5] and independent of activation of Na^+/K^+ adenosine triphosphatase [7]. The stimulatory effect of insulin on Na^+-K^+-$2Cl^-$ cotransport is shown not only in the TAL, but also in BC3H-1 myocytes [8], which has been well characterized for insulin's actions. NaCl absorption in the TAL is known to be stimulated by a number of hormonal effectors that stimulate the production of adenosine 3′,5′-cyclic monophosphate (cAMP)[9]. However, insulin does not affect adenylate cyclase activity in the TAL [9], indicating that cAMP does not fulfill the role of a second messenger in the action of insulin.

The current theory proposes that intrinsic tyrosine kinase activity of the insulin receptor plays a central role in mediating many of insulin's action [10]. Activation of the insulin receptor tyrosine kinase causes phosphorylation of several intracellular substrates such as insulin-receptor substrate-1 (IRS-1) [11]. Phosphorylation of these substrates creates the specific binding site for src homology-2 (SH2) domains in a regulatory subunit (p85) of phosphatidylinositol 3-kinase (PI3-kinase), which phosphorylates the D-3 position of the inositol ring of phosphatidylinositol (PI), PI 4-phosphate (PI4-P), and PI 4,5-bisphosphate (PI4,5-P_2) to produce PI 3-phosphate (PI3-P), PI 3,4-bisphosphate (PI3,4-P_2), and PI 3,4,5-triphosphate (PI3,4,5-P_3), respectively [12]. The activation of PI3-kinase mediates metabolic actions of insulin such as antilipolysis and the stimulation of glucose transport [13, 14]. Another proposed mechanism of insulin's action has been the involvement of protein kinase C (PKC) [15]. Some studies have demonstrated that insulin stimulates diacylglycerol production [16, 17] and increases PKC activity, which subsequently may play an important role in the mediation of insulin's action [18–20]. However, the roles of tyrosine kinase, PI3-kinase, and PKC in insulin's actions in renal tubular transports are not clear.

The purpose of the present study was therefore to determine whether tyrosine kinase, PI3-kinase, and/or PKC regulate insulin-stimulated NaCl absorption in the TAL. To address these issues, we examined the effects of these kinases inhibitors on insulin-stimulated NaCl absorption in the isolated perfused medullary TAL (mTAL).

Methods

In vitro microperfusion of isolated mTALs

Female Japanese White rabbits weighing 1.5 to 2.5 kg were fed a standard laboratory diet and had free access to tap water. On the day of the experiment, they were anesthetized with pentobarbital sodium (35 mg/kg, intravenously), and the left kidney was quickly removed, cut into thin slices, and stored in a chilled dissection solution containing (in mmol/liter) 14 KCl, 44 K_2HPO_4, 14 KH_2PO_4, 9 $NaHCO_3$, and 160 sucrose (pH 7.4). A single mTAL was dissected from the inner stripe of the outer medulla using fine forceps and transferred to a perfusion chamber mounted on an inverted microscope (model IMT-2; Olympus, Tokyo, Japan). The

Received for publication September 22, 1995
and in revised form October 11, 1996
Accepted for publication October 14, 1996

tubule was then perfused according to the *in vitro* microperfusion technique described by Burg and Green [21] with some modifications of Kondo, Yoshitomi and Imai [22]. The perfusion chamber was also perfused at 38°C. Exchange of solutions in the chamber was accomplished by a rotary valve installed beside the microscope, which allowed a rapid exchange of the bath solution, as described by Kondo and Frömter [23]. The perfusate and the bathing solution were identical, and the composition of the solution used in this study was (in mmol/liter) 135 NaCl, 3 KCl, 1.5 $CaCl_2$, 1 $MgCl_2$, 2 KH_2PO_4, 10 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, 5.5 glucose, and 5 L-alanine, adjusted to pH 7.4 by NaOH and gassed with 100% O_2. This study was performed in accordance with the Guide for Animal Experimentation, Tohoku University and Tohoku University School of Medicine.

Measurements of transepithelial voltage

To monitor transepithelial voltage (Vte), the bath solution was connected to a heat-pulled glass capillary that allowed the continuous outflow of 3 mol/liter KCl solution. The KCl solution for the flowing boundary also was connected to the calomel half-cell, which was directly grounded. The perfusion pipette used to monitor Vte was filled with the perfusate, connected to 1 mol/liter KCl agar bridge in a thin polyethylene tube and then to a Ag-AgCl wire. The wire was connected directly to one probe of a high-input impedance electrometer (model FD-223; WPI, New Haven, CT, USA). To avoid interference by the dilution potential of NaCl, the lumina of the mTALs were perfused at more than 20 nl/min.

Measurements of net lumen-to-bath Cl^- flux

Net lumen-to-bath Cl^- flux (J_{Cl}) was determined by electrical measurement with a double-barreled Cl^- microelectrode discribed before [5]. After the luminal fluid was collected from the nonperfused side of the tubule using the collection pipette, a droplet of the solution was transferred to a chamber containing silicon oil, and a double-barreled Cl^- microelectrode was dipped into the droplet to measure Cl^- activity. The electrodes were calibrated to 150, 45, and 15 mmol/liter of the pure NaCl solution at room temperature before and after measuring the collected samples of luminal fluid. Since volume absorption did not differ significantly from zero in this segment, J_{Cl} was calculated from the equation:

$$J_{Cl} = V/L \cdot ([Cl^-]_p - [Cl^-]_c)$$

where V is the perfusion rate, L is tubular length, $[Cl^-]_p$ and $[Cl^-]_c$ are Cl^- activity in perfusate and collected fluid, respectively. In the present study, the perfusion rate in every period of the experiment in which J_{Cl} was measured was approximately 5 nl/min. There was no significant difference in the perfusion rates among the different periods.

Chemicals

Bovine insulin and calphostin C were purchased from Sigma Chemical (St. Louis, MO, USA). IGF-I was kindly provided by Fujisawa Pharmaceutical Co., Ltd. Corning ion exchanger no. 477913 was purchased from Corning Medical (Medfield, MA, USA). Genistein, wortmannin, staurosporin, and all other chemicals were purchased from Wako Pure Chemicals (Osaka, Japan). All inhibitors were dissolved in DMSO at final concentration of less than 0.1%.

Statistical analysis

Data are expressed as the mean ± SE. Statistical comparisons between the control and experimental periods within a group were made using the paired *t*-test, and comparisons between the two groups were made using the unpaired *t*-test. Any value of $P < 0.05$ was accepted as statistically significant.

Results

Effect of insulin on V_{te} and J_{Cl} in the mTAL

Insulin increased dose-dependently V_{te} as we have reported previously [5] and a submaximal stimulatory concentration, that is, 10^{-6} mol/liter insulin was chosen for subsequent experiments. In control experiments, insulin increased V_{te} from 3.1 ± 0.3 to 5.7 ± 0.3 mV ($N = 5$, $P < 0.001$) and J_{Cl} from 143 ± 15 to 292 ± 37 pmol · mm^{-1} · min^{-1} ($N = 5$, $P < 0.005$). To confirm that the stimulatory effect of insulin is mediated through insulin receptor and not that for insulin growth factor-I (IGF-I), the effect of IGF-I on V_{te} was also examined. Vte did not change after a 20 minute incubation of 10^{-7} mol/liter IGF-I (from 3.6 ± 0.4 to 3.3 ± 0.3 mV, $N = 6$, $P > 0.05$).

Effects of tyrosine kinase inhibitors on the action of insulin in the mTAL

We examined the effects of two dissimilar inhibitors of tyrosine kinase on the action of insulin. A total of 148 μmol/liter genistein and 100 μmol/liter methyl 2,5-dihydroxycinnamate were added to the bath 30 minutes prior to incubation with insulin. In the presence of genistein, which is a competitive inhibitor of ATP binding to tyrosine kinase [24], basal V_{te} and J_{Cl} were 2.8 ± 0.4 mV and 140 ± 8 pmol · mm^{-1} · min^{-1}, respectively, and insulin-stimulated Vte and J_{Cl} were 3.2 ± 0.5 mV and 154 ± 15 pmol · mm^{-1} · min^{-1}, respectively. In the presence of methyl 2,5-dihydroxycinnamate, which is a competitive inhibitor of substrate binding to tyrosine kinase [25], basal V_{te} was 2.9 ± 0.2 mV, and insulin-stimulated Vte was 2.9 ± 0.2 mV. Thus, genistein and methyl 2,5-dihydroxycinnamate both abolished the effects of insulin without changing the basal values (Figs. 1 and 2).

Effect of a PI3-kinase inhibitor on the action of insulin in the mTAL

We examined the effect of wortmannin, a specific PI3-kinase inhibitor [26], which was isolated from the culture broth of *Talaromyces wortmanii* KY 12420 [27] on insulin-stimulated V_{te}. One μmol/liter wortmannin was added to the bath 30 minutes prior to incubation with insulin. In the presence of wortmannin, basal V_{te} was 3.4 ± 0.2 mV and insulin-stimulated V_{te} was 4.1 ± 0.2 mV. Thus, wortmannin inhibited the effect of insulin on V_{te} without changing the basal values (Fig. 3).

Effects of PKC inhibitors on the action of insulin in the mTAL

We examined the effects of two dissimilar inhibitors of PKC on insulin-stimulated V_{te} and J_{Cl} · 50 nmol/liter staurosporin and calphostin C were added to the bath 30 minutes prior to incubation with insulin. In the presence of staurosporin, which is a multicyclic microbial product that has been characterized as a potent inhibitor of the catalytic domain of PKC [28], basal V_{te} and J_{Cl} were 3.3 ± 0.4 mV and 152 ± 8 pmol · mm^{-1} · min^{-1}, respectively, and insulin-stimulated V_{te} and J_{Cl} were 3.3 ± 0.4 mV and 154 ± 15 pmol · mm^{-1} · min^{-1}, respectively. In the presence

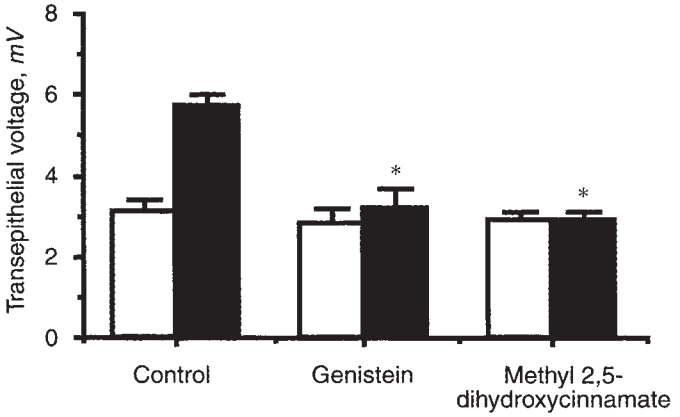

Fig. 1. *Effects of tyrosine kinase inhibitors on insulin-stimulated V_{te} in the mTAL.* A total of 148 μmol/liter genistein and 100 μmol/liter metyl 2,5-dihydroxycinnamate were added 30 minutes prior to an incubation with insulin. Basal values were measured following pretreatment with drugs, and experimental values were measured 20 minutes following the incubation with 10^{-6} mol/liter insulin. Data also were compared to the values in control experiments without drugs. Five tubules were tested in each group. Symbols are: (□) basal; (■) insulin. *$P < 0.05$ versus the values in control experiments.

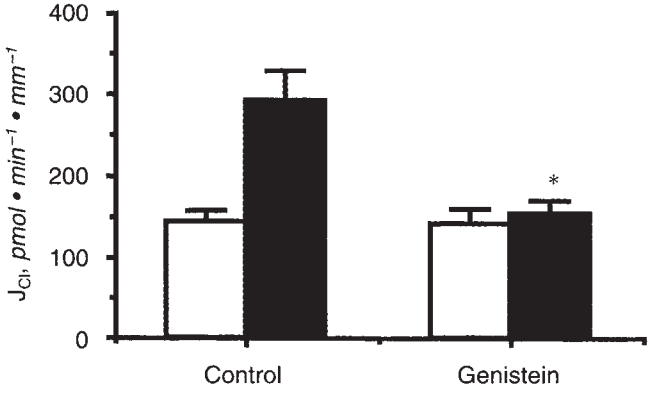

Fig. 2. *Effect of genistein on insulin-stimulated J_{Cl} in the mTAL.* A total of 148 μmol/liter genistein was added 30 minutes prior to incubation with insulin. Collections for basal J_{Cl} determination were made following pretreatment with genistein, and experimental collections were made 20 minutes following an incubation with 10^{-6} mol/liter insulin. Data also were compared to the values in control experiments without genistein. Five tubules were tested in each group. Symbols are: (□) basal; (■) insulin. *$P < 0.05$ versus the values in control experiments.

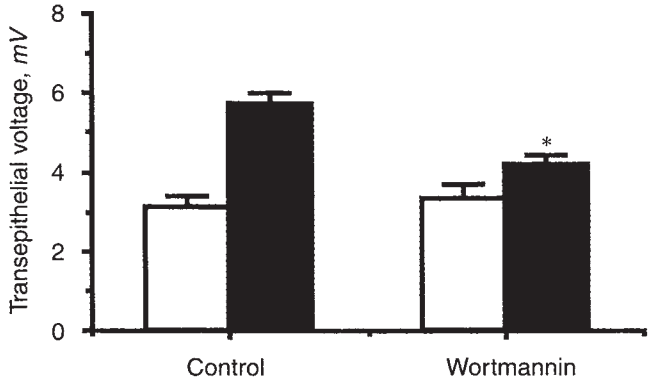

Fig. 3. *Effect of wortmannin on insulin-stimulated Vte in the mTAL.* A total of 1 μmol/liter wortmannin was added 30 minutes prior to incubation with insulin. Basal values were measured following pretreatment with wortmannin, and experimental values were measured 20 minutes following incubation with 10^{-6} mol/liter insulin. Data also were compared to the values in control experiments without wortmannin. Five tubules were tested in each group. Symbols are: (□) basal; (■) insulin. *$P < 0.05$ versus the values in control experiments.

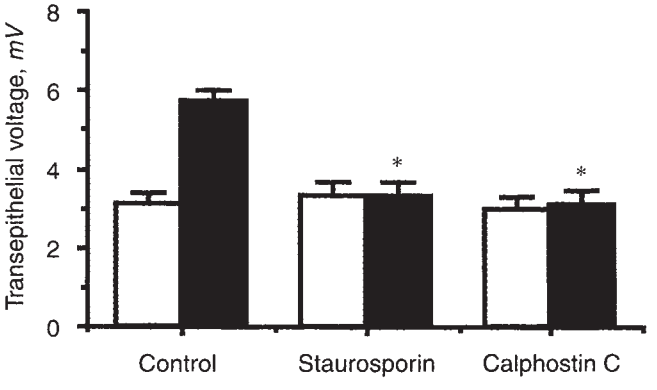

Fig. 4. *Effects of PKC inhibitors on insulin-stimulated V_{te} in the mTAL.* A total of 50 nmol/liter staurosporin and calphostin C were added 30 minutes prior to incubation with insulin. Basal values were measured following pretreatment with drugs, and experimental values were measured 20 minutes following incubation with 10^{-6} mol/liter insulin. Data also were compared to the values in control experiments without drugs. Five tubules were tested in each group. Symbols are: (□) basal; (■) insulin. *$P < 0.05$ versus the values in control experiments.

of calphostin C, which is produced by *Cladosporium cladosporioides* and has been characterized as a potent inhibitor of the regulatory domain of PKC [29], basal V_{te} and J_{Cl} were 3.0 ± 0.3 mV and 145 ± 10 pmol · mm^{-1} · min^{-1}, respectively, and insulin-stimulated V_{te} and J_{Cl} were 3.1 ± 0.4 mV and 150 ± 20 pmol · mm^{-1} · min^{-1}, respectively. Thus, staurosporin and calphostin C inhibited the effects of insulin on V_{te} and J_{Cl} without changing the basal values (Figs. 4 and 5).

Discussion

Our previous study has shown that the stimulatory effect of insulin on NaCl absorption in the mTAL is not mediated by cAMP [5]. The present study has further indicated that insulin stimulates NaCl absorption through tyrosine kinase, PI3-kinase, and PKC-mediated mechanisms.

Genistein and methyl 2,5-dihydroxycinnamate, the specific tyrosine kinase inhibitors, inhibited insulin-stimulated NaCl absorption (Figs. 1 and 2), suggesting that the effect of insulin depends on the receptor's β-subunit tyrosine autophosphorylation or tyrosine kinase substrate phosphorylation. In agreement with our results, genistein inhibits insulin-stimulated Na^+ transport in the A6 cells, which are a distal nephron cell line from *Xenopus laevis* and have properties similar to the mammalian distal nephron [30, 31]. Matsumoto et al [30] have reported that tyrosine kinase inhibitors inhibit also basal Na^+ transport in the A6 cells, suggesting that a tonic tyrosine kinase activity modulates Na^+ transport. Although we used an equivalent concentration of genistein in the present study, we found that genistein did not

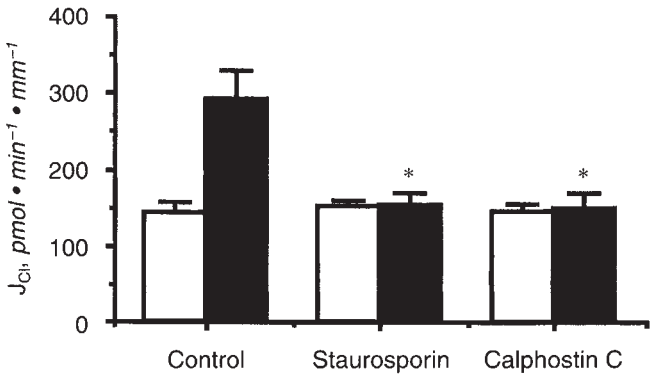

Fig. 5. *Effects of PKC inhibitors on insulin-stimulated J_{Cl} in the mTAL.* A total of 50 nmol/liter staurosporin and calphostin C were added 30 minutes prior to incubation with insulin. Collections for basal J_{Cl} determination were made following pretreatment with drugs, and experimental collections were made 20 minutes following incubation with 10^{-6} mol/liter insulin. Data also were compared to the values in control experiments without drugs. Five tubules were tested in each group. Symbols are: (□) basal; (■) insulin. *$P < 0.05$ versus the values in control experiments.

inhibit basal V_{te} and J_{Cl} in the mTAL (Figs. 1 and 2). This difference may be explained by differences between the cell lines and/or the methods used in these experiments.

Our study demonstrated that the specific PI3-kinase inhibitor wortmannin inhibited insulin-stimulated NaCl absorption (Fig. 3). It has been reported that wortmannin inhibits insulin-induced glucose transport and antilipolysis [13, 14]. Thus far, no study on role of PI3-kinase in insulin's action in ion transports has been published to date. We examined the effect of wortmannin at 1 μmol/liter in the present study. Yano et al reported that 1 μmol/liter wortmannin almost completely inhibited PI3-kinase and myosine light chain kinase, and had no effect on cyclic AMP-dependent protein kinase, cyclic GMP-dependent protein kinase, and Ca^{2+}/calmodulin-dependent protein kinase II, and weakly inhibited PKC to 20% at 10 μmol/liter [26]. The present study shows that PI3-kinase mediates also effects of insulin on ion transports. In agrement with our results, Northern blot analysis demonstrated abundant expression of PI3-kinase mRNA in the kidney [32].

The present study also shows that PKC inhibitors inhibit insulin-stimulated NaCl absorption (Figs. 4 and 5), strongly suggesting that insulin-stimulated NaCl absorption in the mTAL depend on activation of PKC. Both staurosporin and calphostin C completely abolished the effects of insulin at 50 nmol/liter. The concentration of staurosporin here was relatively lower than those reported in studies of insulin-stimulated hexose transport in adipocytes and BC3H-1 myocytes [33]. However, in the latter study, staurosporin was added five minutes prior to treatment with insulin, as opposed to 30 minutes in the present study. While the present study suggests the involvement of PKC in insulin-stimulated NaCl absorption, the possible effect of these PKC inhibitors on other kinases cannot be ruled out. However, two different inhibitors used in the present study showed a similar inhibitory effect on insulin's action. In agreement with our results, PKC inhibitors inhibit insulin-stimulated Na^+ transport in the A6 cell [31] and frog skin [34]. Similarly, phorbol esters stimulate the Na^+-K^+-$2Cl^-$ cotransport in vascular smooth muscle cells [35] and human fibroblasts [36]. On the other hand, the effect of insulin in the isolated perfused mTAL is not mimicked by phorbol ester [37]. In contrast to the single actions, phorbol ester stimulates furosemide-sensitive Na^+ uptake into the mTAL in cell volume changes [38]. Atypical protein kinases C being insensitive to phorbol esters might be involved in insulin's action in the mTAL. At present we have no data to explain the discrepancy.

Because we have reported previously that insulin causes no detectable change in cytosolic free Ca^{2+} in the rabbit mTAL cells [39], the present results also suggest that insulin-induced PKC activation is not related to inositol 1,4,5-triphosphate (IP_3) production. In agreement with our suggestions, Cooper et al reported that insulin increases PKC activity [18]. It is also reported that insulin does not affect inositol phosphates or cytosolic free Ca^{2+} in BC3H-1 myocytes [40]. Three mechanisms of insulin-induced PKC activation have been indicated. The first mechanism is insulin-induced diacylglycerol synthesis [15]. Three potential alternate routes of insulin-induced diacylglycerol synthesis can be considered [15]: (1) *de novo* synthesis from phosphatidic acid [16, 40]; (2) hydrolysis of phosphatidylcholine [16]; and (3) hydrolysis of phosphatidylinositol glycan [17]. These distinct mechanisms can all lead to the production of a structually distinct species of diacylglycerol without IP_3-induced Ca^{2+} mobilization. Second mechanism is the activation of PKC by tyrosine phosphorylation. Insulin stimulates tyrosine phosphorylation of α isoform of classical PKC [41] and δ isoforms of novel PKC [42]. The third mechanism is the activation of PKC by PI3-kinase metabolites. PI3,4-P_2 and PI3,4,5-P_3 were recently reported to activate some isoforms of PKC [43, 44]. Nakanishi, Brewer and Exton reported that PI3,4,5-P_3 activates the ζ isoform of atypical PKC [43]. Toker et al reported that PI3,4-P_2 and PI3,4,5-P_3 activate also δ, ε, and η isoforms of novel PKC [44]. Additionaly, the mRNA of α isoform of classical PKC, δ isoform of novel PKC, and the ζ isoform of atypical PKC are abundantly expressed in the kidney [45]. The probes of *in situ* hybridization to δ isoform of novel PKC and ζ isoform of atypical PKC showed strong labeling in the inner stripe of the outer medulla [45]. It is possible that insulin can cause selective activation of isoforms of PKC and stimulate NaCl transport in the mTAL. Thus far, the effects of insulin on phosphoinositide metabolism, diacylglycerol synthesis and protein kinase C regulation have not been directly examined in the mTAL. To clarify the precise mechanism of insulin's action in the mTAL cells, further studies are necessary.

Acknowledgments

This work was supported in part by a Grant-in-Aid from the Ministry of Education, Science and Culture and by a research grant for cardiovascular diseases (3C-5) from the Ministry of Health and Welfare of Japan. A portion of this study was presented at the 13th International Congress of Nephrology in Madrid, Spain, in 1995. We thank S. Matsumoto and C. Mezawa for their technical assistance.

Reprint requests to Yoshiaki Kondo, M.D., Department of Pediatrics, Tohoku University School of Medicine, Seiryo-machi 1-1, Aoba-ku, Sendai 980, Japan.

References

1. Nizet A, Lefebvre P, Crabre J: Control by insulin of sodium potassium and water excretion by the isolated dog kidney. *Pflügers Arch* 323:11–20, 1971

2. DEFRONZO RA, COOKE CR, ANDERES R, FALOONA GR, DAVIS PJ: The effect of insulin on renal handling of sodium, potassium, calcium, phosphate in man. *J Clin Invest* 55:845–855, 1975
3. DEFRONZO RA, GOLDBERG M, AGUS ZS: The effect of glucose and insulin on renal electrolyte transport. *J Clin Invest* 58:83–90, 1976
4. KIRCHNER KA: Insulin increases loop segment chloride reabsorption in the euglycemic rat. *Am J Physiol* 24:F1206–F1213, 1988
5. ITO O, KONDO Y, TAKAHASHI N, KUDO K, IGARASHI Y, IMAI Y, OMATA K, ABE K: Insulin stimulates NaCl transport in isolated perfused MTAL of Henle's loop of rabbit kidney. *Am J Physiol* 267:F265–F270, 1994
6. MANDON B, SIGA E, CHABARDES D, FIRSOV D, ROINEL N, DE ROUFFIGNAC C: Insulin stimulates Na^+, Cl^-, Ca^{2+}, and Mg^{2+} transports in TAL of mouse nephron. *Am J Physiol* 265:F361–F369, 1993
7. FERAILLE E, MARSY S, CHEVAL L, BARLET-BAS C, KHADOURI C, FAVRE H, DOUCET A: Site of antinatriuretic action of insulin along rat nephron. *Am J Physiol* 263:F175–F179, 1992
8. WEIL-MASLANSKY E, GUTMAN Y, SASSON S: Insulin activates furosemide-sensitive K^+ and Cl^- uptake system in BC3H1 cells. *Am J Physiol* 267:C932–C939, 1994
9. MOREL F: Sites of hormone action in the mammalian nephron. *Am J Physiol* 240:F159–F164, 1981
10. ROSEN OM: After insulin binds. *Science* 237:1452–1458, 1987
11. SUN XJ, ROTHENBERG P, KAHN CR, BACKER JM, ARAKI E, WILDEN PA, CAHILL DA, GOLDSTEIN BJ, WHITE MF: Structure of the insulin receptor substrate IRS-1 defines a unique signal transduction protein. *Nature* 352:73–77, 1991
12. CANTLEY LC, AUGER KR, CARPENTER C, DUCKWORTH B, GRAZIANI A, KAPELLER R, SOLTOFF S: Oncogenes and signal transduction. *Cell* 64:281–302, 1991
13. OKADA T, KAWANO Y, SAKAKIBARA T, HAZEKI O, UI M: Essential role of phosphatidylinositol 3-kinase in insulin-induced glucose transport and antilipolysis in rat adipocytes. *J Biol Chem* 269:3568–3573, 1994
14. HARA K, YONEZAWA K, SAKUE H, ANDO A, KOTANI K, KITAMURA T, KITAMURA Y, UEDA H, STEPHENS L, JACKSON TR, HAWKINS PT, DHAND R, CLARK AE, HOLMAN GD, WATERFIELD MD, KASUGA M: 1-phosphatidylinositol 3-kinase activity is required for insulin-stimulated glucose transport but not for RAS activation in CHO cells. *Proc Natl Acad Sci USA* 91:7415–7419, 1994
15. SALTIEL AR, CUATRECASAS P: In search of a second messenger for insulin. *Am J Physiol* 255:C1–C11, 1988
16. FARESE RV, KONDA TS, DAVIS JS, STANDAERT ML, POLLET RJ, COOPER DR: Insulin rapidly increases diacylglycerol by activating de novo phosphatidic acid synthesis. *Science* 236:586–589, 1987
17. SALTIEL AR, SHERLINE P, FOX JA: Insulin-stimulated diacylglycerol production results from the hydrolysis of a novel phosphatidylinositol glycan. *J Biol Chem* 262:1116–1121, 1987
18. COOPER DR, KONDA TS, STANDAERT ML, DAVIS JS, POLLET RJ, FARESE RV: Insulin increases membrane and cytosolic protein kinase C activity in BC3H-1 myocytes. *J Biol Chem* 262:3633–3639, 1987
19. DRAZMIN B, LEITNER JW, SUSSMAN KE, SHERMAN NA: Insulin and glucose modulate protein kinase C activity in rat adipocytes. *Biochem Biophys Res Commun* 156:570–575, 1988
20. PERSHADSINGH HA, SHADE DL, MCDONALD JM: Insulin-dependent alteration of phorbol ester binding to adipocyte subcellular constitutents. Evidence for the involvement of protein kinase C in insulin action. *Biochem Biophys Res Commun* 145:1384–1389, 1987
21. BURG MB, GREEN N: Function of the thick ascending limb of Henle's loop. *Am J Physiol* 224:659–668, 1973
22. KONDO Y, YOSHITOMI K, IMAI M: Effect of anion transport inhibitors and ion substitution of Cl^- transport in TAL of Henle's loop. *Am J Physiol* 253:F1206–F1215, 1987
23. KONDO Y, FRÖMTER E: Axial heterogeneity of sodium-bicarbonate cotransport in proximal straight tubule of rabbit kidney. *Pflügers Arch* 410:481–486, 1987
24. AKIYAMA T, ISHIDA J, NAKAGAWA S, OGAWARA H, WATANABE S, ITOH N, SHIBUYA M, FUKAMI Y: Genistein, a specific inhibitor of tyrosine-specific protein kinases. *J Biol Chem* 262:5592–5595, 1987
25. UMEZAWA K, HORI T, TAJIMA H, IMOTO M, ISSHIKI K, TAKEUCHI T: Inhibition of epidermal growth factor-induced DNA synthesis by tyrosine kinase inhibitors. *FEBS Lett* 260:198–200, 1990
26. YANO H, NAKANISHI S, KIMURA K, HANAI N, SAITOH Y, FUKUI Y, NONOMURA Y, MATSUDA Y: Inhibition of histamine secretion by wortmannin through the blockade of phospatidylinositol 3-kinase in RBL-2H3 cells. *J Biol Chem* 268:25846–25856, 1993
27. NAKANISHI S, KAKITA S, TAKAHASHI I, KAWAHARA K, TSUKUDA E, SANO T, YAMADA K, YOSHIDA M, KASE H, MATSUDA Y, HASHIMOTO Y, NONOMURA Y: Wortmannin, a microbial product inhibitor of myosin light chain kinase. *J Biol Chem* 267:2157–2163, 1992
28. TAMAOKI T, NOMOTO H, TAKAHASHI I, KATO Y, MORIMOTO M, TOMITA F: Staurosporin, a potent inhibitor of phosholipid/Ca^{++} dependent protein kinase. *Biochem Biophys Res Commun* 135:397–402, 1986
29. KOBAYASHI E, NAKANO H, MORIMOTO M, TAMAOKI T: Calphostin C (UCN-1028C), a novel microbial compound, is a highly potent and specific inhibitor of protein kinase C. *Biochem Biophys Res Commun* 159:548–553, 1989
30. MATSUMOTO PS, OHARA A, DUCHATELLE P, EATON DC: Tyrosine kinase regulates epithelial sodium transport in A6 cells. *Am J Physiol* 264:C246–C250, 1993
31. RODRIGUEZ-COMMES J, ISALES C, KALGHATI L, GASALLA-HERRAIZ J, HAYSLETT JP: Mechanism of insulin-stimulated electrogenic sodium transport. *Kidney Int* 46:666–674, 1994
32. HU P, MONDINO A, SKOLNIK EY, SCHLESSINGER J: Cloning of a novel, ubiquitously expressed human phosphatidylinositol 3-kinase and identification of its binding site on p85. *Mol Cell Biol* 13:7677–7688, 1993
33. STANDAERT ML, BUCKLEY DJ, ISHIZUKA T, HOFFMAN JM, COOPER DR, POLLET RJ, FERASE RV: Protein kinase C inhibitors block insulin and PMA-stimulated hexose transport in isolated rat adipocytes and BC3H-1 myocytes. *Metabolism* 39:1170–1179, 1990
34. CIVAN MM, PETERSON-YANTORNO K, O'BRIEN TG: Insulin and phorbol ester stimulate conductive Na^+ transport through a common pathway. *Proc Natl Acad Sci USA* 85:963–967, 1988
35. OWEN NE: Effect of TPA on ion fluxes and DNA synthesis in vascular smooth muscle cells. *J Cell Biol* 101:454–459, 1985
36. OWEN NE, PRASTEIN ML: Na/K/Cl cotransport in cultured human fibroblasts. *J Biol Chem* 260:1445–1451, 1985
37. NEANT F, IMBERT-TEBOUL M, BAILLY C: Cyclic guanosine monophosphate is the mediator of platelet-activating factor inhibition on transport by the mouse kidney thick ascending limb. *J Clin Invest* 94:1156–1162, 1994
38. EVELOFF J, WARNOCK DG: Activation of ion transport systems during cell volume regulation. *Am J Physiol* 252:F1–F10, 1987
39. ITO O, KONDO Y, TAKAHASHI N, OMATA K, ABE K: Role of calcium in insulin-stimulated NaCl transport in medullary thick ascending limb. *Am J Physiol* 269:F236–F241, 1995
40. FARESE RV, DAVIS JS, BARNES DE, STANDAERT ML, BABISCHKIN JS, HOCK R, ROSIC NK, POLLET RJ: The de novo phospholipid effect of insulin is associated with increases in diacylglycerol, but not inositol phosphates or cytosolic Ca^{2+}. *Biochem J* 231:269–278, 1985
41. LIU F, ROTH RA: Insulin-stimulated tyrosine phosphorylation of protein kinase Ca. *Biochem Biophys Res Commun* 200:1570–1577, 1994
42. LI W, MISCHAK H, YU JC, WANG LM, MUSHINSKI JF, HEIDARAN MA, PIERCE JH: Tyrosine phosphorylation of protein kinase C-δ in response to its action. *J Biol Chem* 269:2349–2352, 1994
43. NAKANISHI H, BREWER KA, EXTON JH: Activation of the ζ isozyme of protein kinase C by phosphatidylinostol 3,4,5-trisphosphate. *J Biol Chem* 268:13–16, 1993
44. TOKER A, MEYER M, REDDY KK, FALCK JR, ANEJA R, ANEJA S, PARRA A, BURNS DJ, BALLAS LM, CANTLEY LC: Activation of protein kinase C family members by the novel polyphoshoinositides PtdIns-3,4-P_2 and PtdIns-3,4,5-P_3. *J Biol Chem* 269:32358–32367, 1994
45. OSTLUND E, MENDEZ CF, JACOBSSON G, FRYCKSTEDT J, MEISTER B, APERIA A: Expression of protein kinase C isoforms in renal tissue. *Kidney Int* 47:766–773, 1995