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Insulin-like growth factor II stimulates production of inositol trisphosphate in proximal tubular basolateral membranes from canine kidney

(brush-border membrane/diacylglycerol/phospholipase C/protein kinase C/somatomedin)

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ABSTRACT **To determine whether insulin-like growth factor II (IGF-II) activates phospholipase C in the basolateral membrane of the renal proximal tubular cell, we incubated basolateral membranes isolated from canine kidney with rat IGF-II (rIGF-II) and measured levels of inositol trisphosphate (Ins-P_3) in suspensions and of diacylglycerol extractable from the membranes. Incubation with rIGF-II increased levels of Ins-P_3 and diacylglycerol in a concentration-dependent manner. Significant enhancement of Ins-P_3 levels and extractable diacylglycerol occurred in suspensions incubated with as little as 10^{-10} M rIGF-II. Elevated levels of Ins-P_3 were measured after as little as 5 sec of incubation. Increases were no longer detectable after 45 sec of incubation, due to dephosphorylation of Ins-P_3 in membrane suspensions. Incubation with either insulin or insulin-like growth factor I did not affect the level of Ins-P_3. IGF-II-stimulated increases in Ins-P_3 did not occur when basolateral membranes were suspended in the absence of free calcium. Increases were demonstrable in basolateral membrane suspensions in 0.1, 0.2, or 0.3 μM calcium, but not in 1.0 μM calcium. Inclusion of guanosine 5′-[γ-thio]triphosphate in incubation mixtures did not increase levels of Ins-P_3, nor did it enhance the action of rIGF-II in this regard. However, inclusion of guanosine 5′-[β-thio]diphosphate inhibited rIGF-II stimulation of Ins-P_3 production. In contrast to findings with basolateral membrane suspensions, incubation with rIGF-II did not increase levels of Ins-P_3 in suspensions of isolated brush-border membranes. Our data are consistent with IGF-II-mediated activation of phospholipase C in isolated proximal tubular basolateral membranes. Such an action could reflect the mechanism by which the IGF-II "signal" is transmitted across the basolateral membrane of the renal proximal tubular cell and by which the actions of this peptide are mediated in renal and non-renal cells.**

Insulin-like growth factors I and II (IGF-I and -II) are peptides with chemical structures similar to insulin. Like insulin, they circulate in plasma and interact with sensitive cells by binding to plasma membrane receptors. As is the case for insulin, the receptor for IGF-I is a tetrameric glycoprotein, consisting of two α (M_r 135,000) and two β (M_r 92,000) subunits. Binding of IGF-I to the α subunit of its receptor has been shown to induce autophosphorylation of the β subunit, which is a tyrosine-protein kinase. It is thought that such autophosphorylation plays a role in transmission of the IGF-I "signal" across the plasma membrane. In contrast to receptors for insulin and IGF-I, the IGF-II receptor is a monomer with $M_r \approx 260{,}000$. No evidence exists to suggest that the IGF-II receptor is a protein kinase (1). The mechanism by which the IGF-II signal is transmitted across the plasma membrane is unknown.

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Previous work in this laboratory characterized separate specific receptors for insulin, IGF-I, and IGF-II in proximal tubular basolateral membranes prepared from canine kidney. Insulin- and IGF-I stimulated phosphorylation of respective β subunits was demonstrable in isolated membranes that had been preincubated with either peptide before exposure to [γ-^{32}P]ATP (2, 3). Preincubation of basolateral membranes with rat IGF-II (rIGF-II) before addition of [γ-^{32}P]ATP resulted in phosphorylation of several basolateral membrane proteins, primarily on serine and threonine residues (4). The identity of the protein kinase that effects IGF-II-stimulated phosphorylation is unknown. However, one possibility is the serine and threonine kinase, protein kinase C, that is present in isolated basolateral membranes (5). Diacylglycerol is the activator of protein kinase C *in vivo* and can stimulate the protein kinase *in vitro*. Diacylglycerol is formed, together with inositol phosphates, as a consequence of the action of phospholipase C on phosphatidylinositol and phosphatidylinositol phosphates present in plasma membranes (6).

We have shown that a calcium-dependent phospholipase C is associated with isolated basolateral membranes. Activity is demonstrable only in the presence of deoxycholate (7). Such localization establishes the potential for IGF-II-stimulated activation of protein kinase C *in vitro* by generation of diacylglycerol effected by phospholipase C.

A variety of hormones and growth factors are known to activate phospholipase C. However, no such effect of an IGF has been described. The initial event in hormonal stimulation that is mediated by phospholipase C is breakdown of phosphatidylinositol 4,5-bisphosphate. The products of this reaction are inositol 1,4,5-trisphosphate (Ins-P_3) and diacylglycerol. The studies reported here were designed to ascertain whether IGF-II activates phospholipase C in isolated proximal tubular basolateral membranes. To this end, we measured generation of Ins-P_3 and diacylglycerol in suspensions of basolateral membranes incubated in the presence or absence of rIGF-II. Our findings are consistent with IGF-II-mediated activation of phospholipase C. Such activation could reflect the mechanism by which IGF-II signal transmission occurs *in vivo*.

METHODS AND MATERIALS

Preparation of Isolated Membranes. Membranes from kidneys of mongrel dogs were used. Suspensions enriched for basolateral membranes originating from proximal tubular cells were prepared by Percoll gradient ultracentrifugation (8). Brush-border membranes were prepared by a $MgCl_2$

Abbreviations: IGF, insulin-like growth factor; rIGF-II, rat IGF-II; Ins-P_3, inositol 1,4,5-trisphosphate; G protein, guanine nucleotide-binding regulatory protein; GTP[γ-S], guanosine 5′-[γ-thio]triphosphate; GDP[β-S], guanosine 5′-[β-thio]diphosphate.

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precipitation technique (9). Membranes were suspended in 250 mM sucrose/100 mM NaCl/50 mM Tris·HCl, pH 7.5, to give a protein concentration of 10 mg/ml and then were frozen at −70°C until used in experiments, as in previous studies (7).

Measurement of Phospholipase C Activity. Phospholipase C activity associated with isolated membranes was evaluated by measuring levels of two products in membrane suspensions after activation of phospholipase C by agonists that effect breakdown of endogenous phosphatidylinositol 4,5-bisphosphate. The products are diacylglycerol, which remains in the membranes, and water-soluble Ins-P_3. Membranes (2 mg of protein) were suspended in 25 mM sucrose/130 mM NaCl/500 μM EGTA/50 mM Tris·HCl, pH 7.5 (no free calcium). The total volume was 500 μl. Where indicated, $CaCl_2$ was included in solutions so as to vary the concentration of free calcium as before (5, 7). Suspensions were incubated for various times in the presence of different concentrations of porcine insulin (Sigma), IGF-I ([Thr-59] IGF-I, Amgen, Thousand Oaks, CA), or rIGF-II [kindly provided by J. Florini and D. Ewton, Syracuse University, Syracuse, NY (10) or by J. R. Gavin III, University of Oklahoma, Oklahoma City, OK (4, 11)]. For most experiments the preparation obtained from J. Florini was used. However, identical results were obtained with the two preparations. Peptides were added to membrane suspensions in 0.01 M sodium acetate, pH 7.5 (1:100 dilution). Alternatively, membranes were incubated in the absence of peptide (sodium acetate added alone). After incubation, membranes were extracted with 1 ml of chloroform/methanol, 2:1 (vol/vol), containing 2.4 M HCl.

To measure Ins-P_3 originating from membranes, water-soluble inositol phosphates contained in the methanol layer of the extraction mixture were separated by HPLC using a Mono-Q HR 5/5 anion-exchange column (Pharmacia), as in the studies of Meek (12). We used a Beckman 421A controller and two Beckman 110B pumps. The mobile-phase gradients were generated from mixtures of buffer A (10 mM Tris·HCl, pH 8.5) and buffer B (500 mM Na_2SO_4/10 mM Tris·HCl, pH 8.5). We employed a linear gradient from 5% to 30% buffer B over 25 min with a flow rate of 1.0 ml/min. With this system, D-[2(n)-^{3}H]inositol 1-phosphate (Amersham, 1 Ci/mmol; 1 Ci = 37 GBq) was eluted in fractions 2–4, D-[2(n)-^{3}H]inositol 1,4-bisphosphate (Amersham, 1 Ci/mmol) was eluted in fractions 10–12, and D-[2(n)-^{3}H]inositol 1,4,5-trisphosphate ([^{3}H]Ins-P_3) (New England Nuclear, 3.6 Ci/mmol) was eluted in fractions 24–26. After HPLC separation of nonradiolabeled inositol phosphates originating from membranes, the Ins-P_3 peak was desalted by passage through a column of Bio-Rex MSZ 501D resin (Bio-Rad). The eluate was concentrated with a Speed-Vac (Savant, Farmingdale, NY). Concentrated samples were suspended in 50 μl of 5 mM $MgCl_2$/50 mM Tris·HCl, pH 9.0, and then were dephosphorylated by addition of 2.5 units of bovine intestinal alkaline phosphatase (Sigma) followed by incubation for 2 hr at 37°C. After incubation, alkaline phosphatase was inactivated by boiling for 3 min and free *myo*-inositol was measured by an enzymatic fluorometric assay (13). Fluorescence was determined with a Farrand fluorometer (Farrand Optical, Mount Vernon, NY). *myo*-Inositol present in experimental samples was determined from a standard curve of known quantities of *myo*-inositol (0.01–0.04 nmol/ml) that encompassed the range of values in experimental samples. Fluorescence was a linear function of *myo*-inositol concentration over this range.

To measure diacylglycerol originating from membranes, the chloroform layer from the extraction mixture was dried under nitrogen, resuspended in 10 μl of chloroform/methanol 2:1, spotted on Whatman LK5D thin-layer chromatography plates (Whatman), and subjected to thin-layer chromatography exactly as before (7). After chromatographic separation, neutral lipids were detected by spraying plates with 10% (wt/vol) $CuSO_4$ in 8% (vol/vol) H_3PO_4, followed by charring. Levels of diacylglycerol extractable from membranes were quantitated by densitometric scanning (7).

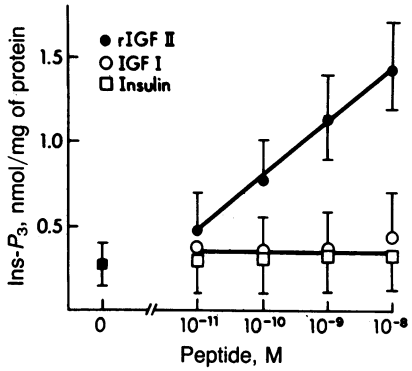

FIG. 1. Levels of Ins-P_3 in basolateral membrane suspensions after 15 sec of incubation with various concentrations of rIGF-II, IGF-I, or insulin or in the absence of peptide. Free calcium concentration was 0.3 μM. Data represent mean ± SEM of 4 experiments.

RESULTS

To determine whether IGF-II activates phospholipase C in proximal tubular basolateral membranes from dog kidney, we first measured levels of Ins-P_3 in membrane suspensions that were incubated in the absence of rIGF-II or in the presence of various concentrations of rIGF-II (10^{-11}–10^{-8} M) for 15 sec prior to extraction. Incubation mixtures contained 0.3 μM free calcium, since we have demonstrated the activity of deoxycholate-stimulated phospholipase C in basolateral membranes at this concentration of calcium (7). Inclusion of rIGF-II in incubation mixtures resulted in a concentration-dependent increase of Ins-P_3 in suspensions. Significantly increased levels, compared to those measured after incubation in the absence of peptide, were detected in suspensions incubated with 10^{-10} M [$P < 0.05$, Dunnett's multiple comparison procedure (14)], 10^{-9} M ($P < 0.01$), or 10^{-8} M ($P < 0.01$) rIGF-II. In contrast to the action of rIGF-II, inclusion of insulin or IGF-I in suspensions did not affect the level of Ins-P_3 (Fig. 1).

Since IGF-II-stimulated breakdown of phosphatidylinositol 4,5-bisphosphate by phospholipase C would be expected to increase levels of diacylglycerol as well as Ins-P_3, we measured diacylglycerol in nonaqueous extracts originating from basolateral membranes that had been incubated with various concentrations of rIGF-II (0–10^{-9} M) under condi-

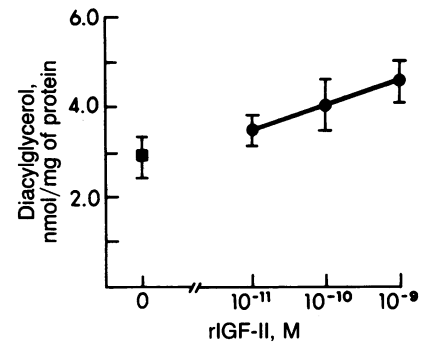

FIG. 2. Diacylglycerol extractable from basolateral membranes incubated with various concentrations of rIGF-II or in the absence of peptide as in Fig. 1. Data represent mean ± SEM of 4 experiments.

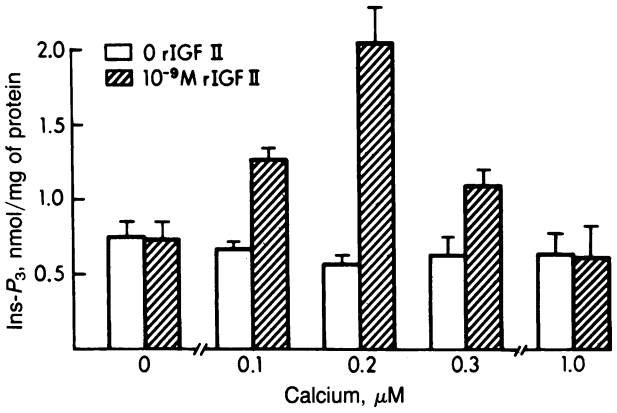

FIG. 3. Levels of Ins-P_3 in basolateral membrane suspensions after 15 sec of incubation with 10^{-9} M rIGF-II or in the absence of peptide. Free calcium was varied as shown. Data represent mean ± SEM of 4 experiments.

tions identical to those employed to generate the data shown in Fig. 1. As shown in Fig. 2, inclusion of rIGF-II in incubation mixtures increased extractable diacylglycerol in a concentration-dependent manner. Significant enhancement was observed after incubation of basolateral membranes with 10^{-10} or 10^{-9} M rIGF-II ($P < 0.05$ for each, Dunnett's multiple comparison procedure). The quantities of diacylglycerol extractable from membranes incubated with rIGF-II minus the quantities extractable from membranes incubated without rIGF-II (net IGF-II-stimulated increase in diacylglycerol) were not significantly different from the net IGF-II-stimulated increase in Ins-P_3. Thus, the data of Figs. 1 and 2 are consistent with rIGF-II-stimulated hydrolysis of phosphatidylinositol 4,5-bisphosphate in isolated basolateral membranes resulting in formation of Ins-P_3 and diacylglycerol.

To determine whether rIGF-II-mediated increases of Ins-P_3 levels are affected by the concentration of free calcium in basolateral membrane suspensions, we measured Ins-P_3 after incubation of membranes for 15 sec in the absence or presence of rIGF-II (10^{-9} M) with 0, 0.1, 0.2, 0.3, or 1.0 μM free calcium (Fig. 3). Changes in calcium over this range did not affect levels of Ins-P_3 in membrane suspensions incubated in the absence of rIGF-II. In the presence of 10^{-9} M rIGF-II, increased levels of Ins-P_3 were measured in suspensions incubated with 0.1 μM ($P < 0.05$, paired Student's t test), 0.2 μM ($P < 0.01$) or 0.3 μM ($P < 0.05$) calcium but not with 0 or 1.0 μM calcium. These findings indicate that the action of rIGF-II to increase Ins-P_3 is a calcium-dependent process, but the absence of an effect at 1.0 μM calcium suggests that the action is demonstrable over only a limited range of calcium concentration. Concentrations from 0.1–0.3 μM are characteristic of intracellular calcium in renal cells (15). Subsequent incubations were carried out in 0.2 μM calcium.

We next determined the levels of Ins-P_3 in suspensions of basolateral membranes incubated with 10^{-9} M rIGF-II for various times. Increased Ins-P_3 was detectable after as little as 5 sec of incubation with rIGF-II but no longer detectable after 45 sec of incubation (Fig. 4). This suggests that both formation and breakdown of Ins-P_3 occur in suspensions and that either the stimulus for formation is short-lived or the availability of substrate (phosphatidylinositol 4,5-bisphosphate) is limiting so that all the Ins-P_3 formed is broken down by 45 sec. To demonstrate breakdown directly, we incubated basolateral membranes with [^{3}H]Ins-P_3 for 15 sec or 1 min and determined the distribution of radioactivity. Fig. 5 shows

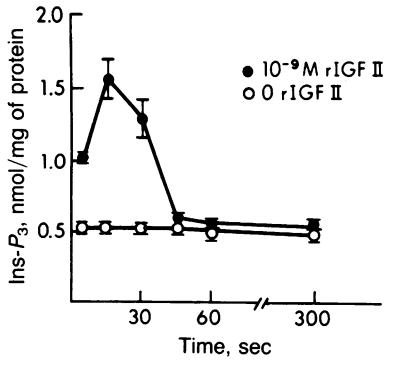

FIG. 4. Levels of Ins-P_3 in basolateral membrane suspensions after various times of incubation with 10^{-9} M rIGF-II or in the absence of peptide. Free calcium concentration was 0.2 μM. Data represent mean ± SEM of 3 experiments.

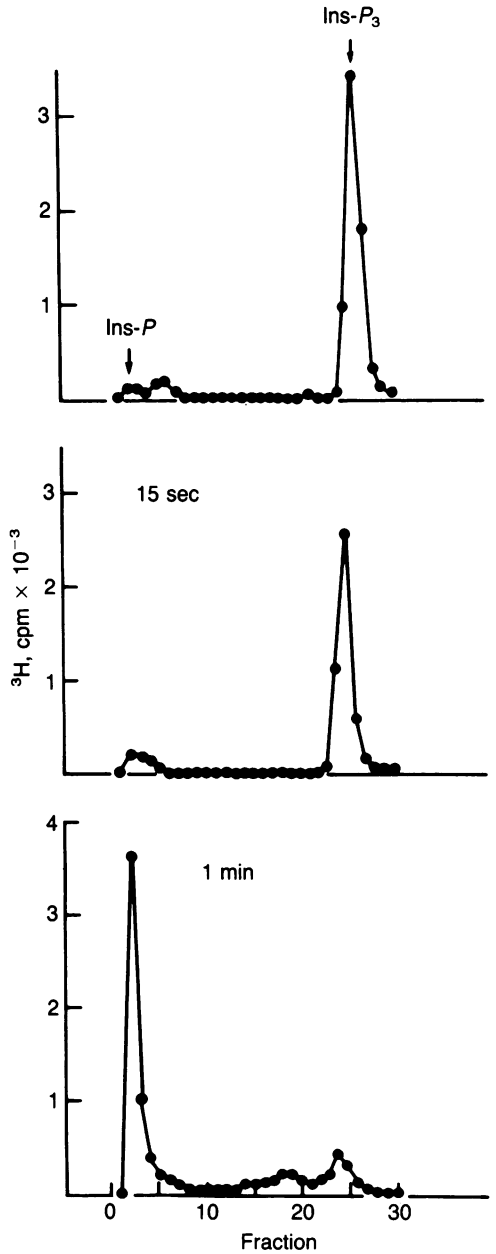

FIG. 5. Radiolabeled inositol phosphates in basolateral membrane suspensions incubated with [^{3}H]Ins-P_3 for 15 sec (*Middle*) or 1 min (*Bottom*). Free calcium concentration was 0.2 μM. After incubations, supernatants were subjected to HPLC separation. Also shown is an HPLC separation of radiolabel not incubated with membranes (*Top*). The positions of authentic inositol 1-phosphate (Ins-*P*) and Ins-P_3 are indicated. Data are from a representative experiment. Recoveries of injected radioactivity were >90%.

that breakdown of Ins-P_3 to inositol phosphate does occur in basolateral membrane suspensions. During 15 sec of incubation in 0.2 μM calcium, 28.8 ± 1.4% of Ins-P_3 was dephosphorylated (n = 3 experiments). The extent of dephosphorylation measured at 15 sec was not greater in the absence of free calcium (13.6 ± 7.0%) or in the presence of 1 μM free calcium (20.9 ± 2.3%). Thus, enhanced Ins-P_3 breakdown cannot explain the effects of 0 or 1.0 μM calcium on levels of Ins-P_3 shown in Fig. 3. Breakdown of added [^{3}H]Ins-P_3 was virtually complete by 60 sec, corresponding to the time course shown in Fig. 4.

A guanine nucleotide-binding regulatory protein (G protein) has been postulated to participate in agonist-mediated activation of phospholipase C (16). To determine whether the actions of rIGF-II are affected by inclusion of guanine nucleotide in incubations, basolateral membranes were incubated for 15 sec in the absence or presence of 10^{-9} M rIGF-II with or without 10 μM guanosine 5'-[γ-thio]triphosphate (GTP[γ-S]), a nonhydrolyzable GTP analog. Addition of GTP[γ-S] to suspensions did not increase levels of Ins-P_3 in the absence or presence of rIGF-II (Fig. 6 *Upper*). This could indicate that the effect of IGF-II in basolateral membranes is mediated independently of a G protein. Alternatively, sufficient GTP may be associated with isolated membranes so as to mask any action of exogenous GTP[γ-S]. To distinguish between these possibilities we determined whether the effect of IGF-II to increase levels of Ins-P_3 is blunted by guanosine 5'-[β-thio]diphosphate (GDP[β-S]), a nonhydrolyzable GDP analog that has been shown to inhibit guanine nucleotide-dependent activation of adenylate cyclase (17) and phospholipase C (18). Significant stimulation of Ins-P_3 production by rIGF-II was not observed in the presence of GDP[β-S] (Fig. 6 *Lower*). Levels of Ins-P_3 in basolateral membrane suspensions incubated with rIGF-II alone were significantly greater than levels in suspensions incubated with rIGF-II and GDP[β-S]. Thus, inclusion of GDP[β-S] inhibits IGF-II-mediated activation of phospholipase C. These findings suggest that a G protein does participate in the activation of phospholipase C by IGF-II.

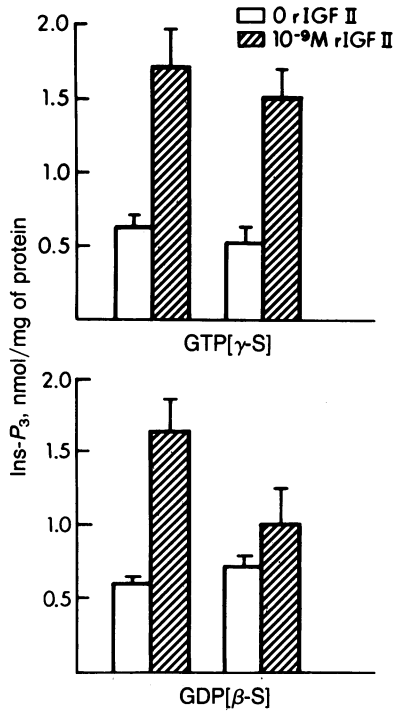

FIG. 6. Levels of Ins-P_3 in basolateral membrane suspensions after 15 sec of incubation with 10^{-9} M rIGF-II or in the absence of peptide. Where noted, 10 μM GTP[γ-S] or 500 μM GDP[β-S] was included in incubation mixtures. Free calcium concentration was 0.2 μM. Data represent mean + SEM of 4 experiments each for *Upper* and *Lower* (a total of 8 experiments). (*Upper*) Levels of Ins-P_3 in suspensions incubated with rIGF-II were significantly higher than levels in suspensions incubated without rIGF-II in the absence ($P <$ 0.05, paired Student's t test) or presence ($P < 0.05$) of GTP[γ-S]. (*Lower*) Levels of Ins-P_3 in suspensions incubated with rIGF-II were significantly higher than levels in suspensions incubated without rIGF-II in the absence ($P < 0.01$, Dunnett's multiple comparison procedure) of GDP[β-S] but not in the presence of GDP[β-S]. Levels in suspensions incubated with rIGF-II alone were significantly higher than levels in suspensions incubated with rIGF-II and GDP[β-S] (P < 0.05).

We have shown that deoxycholate-activated, calcium-dependent phospholipase C is asymmetrically distributed in the plasma membrane of the renal proximal tubular cell, being present in basolateral membranes but not in brush-border membranes (7). In contrast, receptors for IGF-II are present in both membranes (19). To investigate the distribution of IGF-II-stimulated phospholipase C activity, we incubated basolateral and brush-order membranes under identical conditions in the absence or presence of 10^{-9} M rIGF-II for 15 sec and measured Ins-P_3 extractable from suspensions. In contrast to the action of rIGF-II in basolateral membrane suspensions, levels of Ins-P_3 extractable from brush-border membranes were not affected by inclusion of rIGF-II in incubation mixtures (Fig. 7). This finding is consistent with an asymmetrical distribution of IGF-II-stimulated phospholipase C, activity being present in the basolateral membrane but not in the brush-border membrane.

DISCUSSION

Little is known about the mechanism(s) by which the IGF-II signal is transmitted across the plasma membrane of sensitive cells. To learn more about this process in kidney, we characterized binding of rIGF-II to plasma membranes of renal proximal tubular cells from canine kidney (19). Specific binding of ^{125}I-labeled IGF-II was demonstrable in isolated basolateral and brush-border membranes with 50% maximal binding capacities of 3 × 10^{-9} M for each membrane. ^{125}I-labeled IGF-II bound to a protein of M_r 260,000 (19). These findings indicated that receptors for IGF-II are present in proximal tubule that are similar to receptors described in non-renal cells (1). These results established the potential to study the mechanism of IGF-II action at this site.

A variety of polypeptide growth factors act to stimulate Na^+/H^+ exchange across the plasma membrane of target

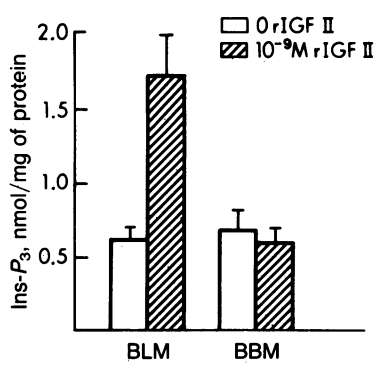

FIG. 7. Levels of Ins-P_3 in basolateral (BLM) and brush-border (BBM) membrane suspensions after 15 sec of incubation with 10^{-9} M rIGF-II or in the absence of peptide. Free calcium concentration was 0.2 μM. Data represent mean + SEM of 4 experiments. Levels of Ins-P_3 in basolateral membrane suspensions incubated with rIGF-II were significantly higher than levels in suspensions incubated in its absence ($P < 0.05$, paired Student t test).

cells, resulting in intracellular alkalinization. Changes in levels of Na^+ and/or H^+ resulting from this process are postulated to mediate effects of these peptides (20). To determine whether IGF-II acts in this manner, proximal tubular segments from canine kidney were incubated with rIGF-II and changes of intracellular pH were measured (11). rIGF-II effected alkalinization of cells within the segments by stimulating Na^+/H^+ exchange across the brush-order membrane. Alkalinization could be demonstrated following incubation with as little as 10^{-11} M rIGF-II. Neither insulin nor IGF-I exerted a similar effect, consistent with alkalinization being mediated by the IGF-II receptor (11). It was postulated that enhanced Na^+/H^+ exchange in isolated segments reflects an action of IGF-II to alkalinize proximal tubular cells *in vivo*.

To provide insight into the mechanism by which the IGF-II signal is transmitted in proximal tubule, experiments were performed to ascertain whether IGF-II stimulated phosphorylation of proteins in proximal tubular membranes. Preincubation of basolateral, but not brush-border, membranes with rIGF-II prior to exposure to [γ-^{32}P]ATP resulted in phosphorylation of several membrane-associated proteins. Incorporation of ^{32}P into the most prominently phosphorylated protein was enhanced significantly by preincubation with as little as 10^{-11} M rIGF-II. Phosphorylation occurred predominantly on serine and threonine residues (4). We subsequently determined that protein kinase C is present in isolated basolateral and brush-border membranes from canine kidney (5) and that deoxycholate-activated, calcium-dependent phospholipase C is localized to the basolateral membrane (7). These findings suggested a sequence of events to explain IGF-II-stimulated phosphorylation of basolateral membranes. In this schema, IGF-II acting to stimulate phospholipase C would generate diacylglycerol to activate protein kinase C and effect phosphorylation of basolateral membrane proteins. Stimulation of phospholipase C could also explain rIGF-II-induced alkalinization of cells within proximal tubular segments. Protein kinase C-mediated phosphorylation of proximal tubular brush-border membranes has been shown to enhance Na^+/H^+ exchange (21). Tumor-promoting phorbol esters, known activators of protein kinase C, stimulate Na^+/H^+ exchange in cells within canine proximal tubular segments (22). IGF-II-induced generation of diacylglycerol at the inner leaflet of the basolateral membrane lipid bilayer, followed by diffusion of diacylglycerol to the brush-border membrane, could activate membrane-associated or cytosolic protein kinase C at this site in intact cells and effect stimulation of the Na^+/H^+ exchanger. A model for such diffusion of diacylglycerol has been presented by Dragsten *et al.* (23).

The present studies were performed to test directly the hypothesis that IGF-II activates phospholipase C in the basolateral membrane of the renal proximal tubular cell. We found that incubation of basolateral membrane suspensions with as little as 10^{-10} M rIGF-II effects formation of Ins-P_3 and diacylglycerol. Our observations are consistent with IGF-II-mediated activation of phospholipase C in the basolateral membrane. The inability to effect formation of Ins-P_3 by incubation of basolateral membranes with as much as 10^{-8} M insulin or IGF-I suggests that the actions of IGF-II are not mediated by receptors for insulin or IGF-I. Activation of phospholipase C in isolated basolateral membranes could reflect the mechanism by which IGF-II signal transduction occurs in kidney and in non-renal cells.

Phospholipase C-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate in intact cells results in increased levels of intracellular calcium originating from intracellular stores and/or from extracellular sources (16). Nishimoto *et al.* have shown (24) that rIGF-II stimulates calcium influx in BALB/c 3T3 cells primed with epidermal growth factor. These investigators postulated that the action of rIGF-II is mediated by a G protein because it was abolished by pretreatment of cells with pertussis toxin, a known modifier of G proteins, and because binding of IGF-II to BALB/c 3T3 cells was inhibited in a concentration-dependent manner by GTP[γ-S] (24). In view of our findings with basolateral membranes, it is possible that IGF-II-stimulated calcium influx in BALB/c 3T3 cells is linked to activation of phospholipase C.

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