D4 dopamine receptor enhances angiotensin II-stimulated aldosterone secretion through PKC-ε and calcium signaling

Hong-Wei Chang,1 Vin-Cent Wu,1 Chao-Yuan Huang,2 Hong-Yu Huang,1 Yung-Ming Chen,1 Tzong-Shinn Chu,1 Kwan-Dun Wu,1 and Bor-Shen Hsieh1

1Nephrology Division, Department of Internal Medicine; and 2Department of Urology, National Taiwan University Hospital, Taipei, Taiwan

Submitted 11 October 2007; accepted in final form 11 December 2007

Chang H-W, Wu V-C, Huang C-Y, Huang H-Y, Chen Y-M, Chu T-S, Wu K-D, Hsieh B-S. D4 dopamine receptor enhances angiotensin II-stimulated aldosterone secretion through PKC-ε and calcium signaling. Am J Physiol Endocrinol Metab 294: E622–E629, 2008. First published January 2, 2008; doi:10.1152/ajpendo.00657.2007.—Aldosterone secretion is subjected to dopaminergic regulation. Our previous study showed that both human D2 and D4 dopamine receptors (D2R and D4R) modulate aldosterone secretion, but in opposing directions. The inhibitory effect of D2R is mediated by attenuating protein kinase C-μ (PKC-μ) and calcium-dependent signaling. The mechanism of D4R effect on angiotensin II (AII)-stimulated aldosterone secretion is explored in this study. Experiments were done with primary human adrenal cortical cells and human adrenocarcinoma (NCI-H295R) cells. Activation of different PKC isoforms was detected by specific phospho-PKC antibodies and PKC translocation. The role of calcium-dependent signaling was examined by measuring the cytoplasmic inositol 1,4,5-triphosphate (IP3) and calcium ([Ca2+]i). We conclude that D4R enhances AII-stimulated aldosterone secretion and secretion as early as 30 min following exposure independently of the modulation of aldosterone synthase (CYP11B2) transcription. CYP11B2 mRNA level elevated by AII was augmented by D4R in the later period. These effects were reversed by the D4R antagonist L-745,870. All activated PKC-α/βI, ε, and μ but not PKC-βII, γ, or -δ of H295R cells. The D4R agonist selectively enhanced AII-stimulated PKC-ε phosphorylation and its translocation to the cell membrane. Furthermore, the D4R agonist enhanced the AII-stimulated elevation of intracellular IP3 and [Ca2+]i. Inhibition of PKC-ε translocation by the PKC-ε-specific inhibitory peptide attenuated AII-stimulated aldosterone secretion, CYP11B2 mRNA expression, and elevation of intracellular IP3 and [Ca2+]i. We conclude that D4R augmented aldosterone synthesis/secrection induced by AII. The mechanisms responsible for this augmentation are mediated through enhancing PKC-ε phosphorylation and [Ca2+]i elevation.

D4R-resistant aldosterone-producing adenoma; protein kinase C-ε; hypertension

There is increasing evidence (8, 12) that aldosterone plays a direct role in the pathogenesis of chronic heart failure and vascular inflammation. The vascular and perivascular inflammatory responses to angiotensin II (AII) infusion and salt loading, both reported to increase cardiovascular aldosterone synthesis (28, 29), are completely prevented by adrenalectomy (25). This suggests that the regulation of adrenal aldosterone production is more important than that of local cardiac and/or vascular synthesis of aldosterone. Although the regulation of aldosterone production by AII is well established, the modulating factors affecting itself or its downstream signaling are controversial and far from being completely delineated. The presence of chromaffin cells originating from the medulla in the cortical layers is strong evidence supporting the concept of the neurohormonal control of zona glomerulosa cell secretion (5).

Dopamine D2-like receptors have been found (9, 19) to play a pivotal role in inhibiting aldosterone secretion. We (31) and other investigators (23) have demonstrated that two subtypes of dopamine receptors, D2 and D4 receptors (D2R and D4R), are expressed in the adrenal cortex, mainly in the zona glomerulosa, and exert opposite effects on aldosterone secretion. The D2R attenuates AII-induced secretion of aldosterone, whereas the D4R enhances that effect.

An increase of intracellular Ca2+ concentration ([Ca2+]i) plays as an important signal in AII-induced aldosterone secretion and CYP11B2 mRNA expression (21, 32), and involvement of protein kinase C (PKC) has also been noted (2, 3, 17) in the regulation of adrenal steroidogenic genes. Our recent work (6) has revealed that D2R modulates AII-stimulated aldosterone synthesis and secretion through attenuation of PKC-μ phosphorylation and [Ca2+]i elevation. Paradoxically, constitutive activation of some subtypes of PKC has been shown (15, 16) to inhibit AII-stimulated CYP11B2 gene expression. Therefore, the expression of the CYP11B2 gene may be differentially regulated by different PKC subtypes (16). In the present study, we explored the role of D4R and its signals in the regulation of AII11B2 mRNA expression and aldosterone secretion of human adrenal cortical cells. We found that D4R could augment both acute and chronic phases of aldosterone secretion/synthesis by AII through specific activation of the novel PKC-ε and [Ca2+]i elevation.

MATERIALS AND METHODS

Materials. All, PD-168,077, and L-745,870 were purchased from Sigma Chemical (St. Louis, MO), and phospho-PKC-specific antibodies and PKC subtype-specific antibodies were purchased from Cell Signaling Technology, (Beverly, MA). PD-168,077 and L-745,870 are the highly selective agonist and antagonist, respectively, for D4R (10, 20). PKC-ε translocation inhibitor peptide and PKC-ε translocation inhibitor peptide-negative controls were obtained from Calbiochem (Cambridge, MA). An inositol 1,4,5-triphosphate (IP3) radioreceptor assay (NEK064) kit was bought from PerkinElmer Life Sciences (DuPont-New England Nuclear, Boston, MA). Fura 2-AM, Pluronic F-127 was obtained from Molecular Probes (Eugene, OR). Angiotensin type 1 receptor (AT1R) antibody and its immunizing peptide (AT1N10 and sc1173, respectively) were obtained from Santa Cruz

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Biotecnvity (Santa Cruz, CA). D4R antibody was designed to target the third intracellular domain. The antibody was purified from the serum of a rabbit immunized with the synthesized oligopeptide RAPPRGPSGPPSPT at the aa233–247 position. The preimmunized serum of the same rabbit was also collected as a negative control. The antibody detected a single band, 46 kDa in size, in both the adrenal cortex, primary human adrenal cortical cells, and H295R cells.

Cell culture. The human adrenocortical carcinoma cell line H295R was obtained from American Type Culture Collection (Rockville, MD). The primary human adrenal cortical cells were prepared from surgical specimens of the patients without any adrenal disease. The primary culture was prepared as described previously (18). In brief, small pieces of normal tissues were dissociated with 0.3% collagenase I A and 20 mg/ml deoxyribonuclease I (Sigma Chemical) in culture medium at 37°C. The digestion was carried out during two 2-h periods for normal, adjacent adrenals. The dispersed cells were washed once with medium and plated on six-well plastic cell culture dishes. The cells were maintained in Dulbecco’s Modified Eagle’s Medium-Ham’s F-12 medium containing 10% fetal calf serum, 2 mmol/l glutamine (Gibco, Grand Island, NY), 1.25 × 10^−5 IU/ml penicillin, and 0.125 g/l streptomycin sulfate (Gibco). The cultures were maintained at 37°C in humidified 95% air-5% CO₂, with replacement of medium every 3rd day until they had achieved subconfluency on 10 cm dishes. The total volume of 20 ml in the culture dish comprised of 100 ng of template cDNA. All samples were tested in a Quantitect system (QIAGEN, Hilden, Germany). Assay no. Hs99999905_m1 was used as an endogenous control in the human adrenal cortex, primary human adrenal cortical cells, and H295R cells.

Measurement of aldosterone. The culture supernatant and cell lysate were collected 30 min or 24 h after treatment, respectively. The aldosterone levels of the culture medium were measured by radioimmunoassay with commercial kits (Aldosterone Maia kit; Biochem Immunosystems, Bologna, Italy).

Quantitative real-time PCR. Total RNA was extracted from H295R cells using a Trizol RNA isolation reagent (Invitrogen) according to the manufacturer’s instructions. RNA was subjected to deoxyribonuclease treatment using 1 U deoxyribonuclease I (amp grade)/μg RNA incubated in deoxyribonuclease reaction buffer for 15 min at 37°C (New England Bilolabs, Beverly, MA) to remove genomic DNA contamination. The reaction was stopped by heating to 90°C for 5 min. RNA was reverse transcribed by using a reverse transcription system kit (Promega, Madison, WI) as described previously (31). The cells expressed D4R and significantly secreted aldosterone after addition of AII. After the doses of the drugs that regulate aldosterone secretion/synthesis were tested. 10 mmol/l AII, 10^−6 mol/l PD-168,077, and 10^−6 mol/l L-745,870 were used in all of the experiments in the present study, unless otherwise indicated. All experiments were performed at least in triplicate; for each experiment the data for analysis was the mean of three measured samples. The study was approved by the Ethics Committee of the National Taiwan University Hospital.

Immunoblotting. For PKC assays, H295R cells were scraped 5 min after treatment and solubilized in lysis buffer. Equal amounts of protein (40 μg for H295R cells) were separated on a 10% polyacrylamide gel and transferred to Immobilon P. Blots were probed with different antibodies, followed by a horseradish peroxidase-coupled anti-rabbit secondary antibody. Immunoreactive proteins were visualized with enhanced chemiluminescence (Pierce, Rockford, IL).

Cytoplasmic Ca²⁺ measurement. The cells were suspended in PBS containing 2 mmol/l EDTA by periodic shaking, washed in a Ca²⁺-containing solution (140 mmol/l NaCl, 5 mmol/l KCl, 1 mmol/l MgCl₂, 1 mmol/l CaCl₂, 11 mmol/l NaHCO₃, 10 mmol/l glucose, 10 mmol/l HEPES, pH 7.4, and 0.1% BSA), and incubated in 4 μM fura 2-AM with 0.04% Pluronic F-127 for 35 min. Cells were then washed and resuspended in the Ca²⁺-containing solution for the following experiments. Intracellular Ca²⁺ was measured in cells suspended in Ca²⁺-containing solution at the ratio of fluorescence with 340- and 380-nm excitation and 510-nm emission (fluorolog 2; Spex Industries, Edison, NJ). The fluorescent ratio was calibrated by adding digitonin to a final concentration of 75 μg/ml and then adding 1 nM EDTA at a 1:50 dilution and 10 N NaOH at a 1:700 dilution, and [Ca²⁺]i was calculated as described (8).

Determination of IP₃. Cells were washed twice and preincubated for 30 min at 37°C in an incubation buffer (145 mmol/l NaCl, 5.6

55kDa+ 43kDa+ 36kDa+

D4R

AT1R

Fig. 1. Expression of D4 receptor (D4R) and angiotensin type I receptor (AT1R) in human adrenal cortex (lane 1), primary cultured cells of human adrenal cortex (lane 2), and H295R cells (lanes 3–5). Immunoblotting with specific antiserum to D4R or AT1R (lanes 1–3) revealed 46- and 42-kDa-sized bands, respectively. The bands were almost absent when the primary antibodies were neutralized with their respective immunizing peptides (lane 4) or when preimmunized serum in place of the D4R antiserum was used (lane 5.)
mmol/l KCl, 5.6 mmol/l glucose, 0.01% BSA, and 10 mmol/l HEPES, pH 7.4). After treatment in 1 ml of warmed incubation buffer for 10 s, 250 μl of ice-cold perchloric acid (10% vol/vol) was added to terminate the response. After scraping, the cells were washed with 250 μl of 10% perchloric acid and centrifuged at 12,000 g for 5 min at 4°C. The supernatants were neutralized with 50 μl of 1.5 M NaOH in the presence of universal indicator. IP3 levels were measured by a specific, competitive binding assay kit (PerkinElmer Life Sciences). Each incubation contained 500 μl of receptor preparation/[3H]IP3 tracer 1:15 (vol/vol), 100 μl of standard IP3 (0–120 pmol/0.1 ml), or cell extract. The tubes were agitated and incubated for 45 min on ice. Incubations were terminated by centrifugation at 12,000 g for 5 min at 4°C. The supernatant was removed by aspiration, and the pellet was dissolved in scintillation liquid and counted.

Statistics. Statistical analysis was performed with the Mann-Whitney U-test using the Statview software package (Abacus Concept, Berkeley, CA). Statistical significance was considered at the 5% level.

RESULTS

D4 receptor was expressed on both human adrenal cortex and H295R cells. In a previous study (31), we demonstrated mRNA expression of human D2R and D4R in adrenal cortex and NCI-H295R (H295R) cells. Immunoblotting with the D4 receptor-specific antibody revealed a single band of 46 kDa in the human adrenal cortex, primary human adrenal cortical cells, and H295R cells (Fig. 1). The band was almost abolished by using preimmunized serum as the primary antibody or by pretreating the anti-D4R serum with the D4R-immunizing peptide.

D4 receptor modulated All-stimulated CYP11B2 mRNA expression and aldosterone secretion. The D4R agonist PD-168,077 (10⁻⁶ mol/l) augmented the increase of All-stimulated 24-h aldosterone secretion of both primary human adrenal cortical cells and H295R cells. This enhancing effect of the D4R was reversed by the D4 antagonist L745,870 (Fig. 2, A and B, top). The effects of both PD-168,077 and L-745,870 were concentration dependent from 10⁻⁸ to 10⁻⁶ mol/l (data not shown). Consistent with its effect on All-stimulated aldosterone secretion, PD-168,077 at 10⁻⁶ mol/l effectively augmented All-stimulated CYP11B2 mRNA expression as early as 2 h after exposure and for ≤24 h (data not shown), and L-745,870 reversed the effect of PD-168,077. The CYP11B2 mRNA expression of primary human adrenal cortical cells and H295R cells 4 h after treatment was illustrated (Fig. 2, A and B, bottom).

![Fig. 2. Modulation of D4R on angiotensin II (All; at 10⁻⁸ mol/l)-stimulated aldosterone secretion and expression of CYP11B2 mRNA from primary cultured cells of human adrenal cortex (A and C) and H295R cells (B and D). A and B: aldosterone secretion 24 h after treatment (top) and CYP11B2 mRNA expression 4 h after treatment (bottom). *Significantly different from All (P < 0.05). C and D: aldosterone level of the supernatant (solid bars) and the intracellular compartment (hatched bars) 30 min after treatment. †Significantly different from All (P < 0.05). Cont, control; PD, PD-168,077 at 10⁻⁶ mol/l; L, L-745,870 at 10⁻⁶ mol/l. All experiments were performed in at least triplicate; for each experiment the data for analysis were the mean of 3 measured samples. Values are shown as means ± SD.](http://ajpendo.physiology.org/)
The augmented effect of PD-168,077 on AII-stimulated aldosterone secretion of both primary human adrenal cortical cells and H295R cells was observed as early as 30 min after exposure and could be reversed by L-745,870 (Fig. 2, C and D). In addition to the elevation of aldosterone levels in the supernatant, the intracellular aldosterone level was also increased by AII, which was augmented by PD-168,077, and L-745,870 reversed the effect of PD-168,077. There was no significant change of CYP11B2 mRNA level 30 min after treatment with AII (data not shown).

D4R altered the AII-stimulated PKC response. Phospho-PKC-specific antibodies showed that AII stimulated phosphorylation of PKC isoforms 5 min after treatment in primary human adrenal cortical cells (A) and H295R cells (B) was detected with phospho-PKC-specific antibodies. Quantification of phospho-PKC-ε on immunoblotting was measured by densitometer. *Significantly different from AII (P < 0.05). C: membrane translocation of PKC isoforms 5 min after treatment in H295R cells. Both the membrane (M) and cytosol proteins (C) were immunoblotted with phospho-PKC-specific antibodies. †Significantly different from AII (P < 0.05). The quantitative change of PKC-ε in the membrane (solid bar) and cytosol (hatched bars) is shown in the bar graph. All experiments were performed in at least triplicate; for each experiment the data for analysis were the mean of 3 measured samples. Values are shown as means ± SD.
lation of PKC-α/βII Thr638/641, PKC-ε Ser729, PKC-μ Ser744/748, and PKC-μ Ser744/748 but not of PKC-δ Thr505, PKC-θ Thr538, or PKC-ζ/η Thr410/403, on both primary human adrenal cortical cells and H295R cells (6). Addition of PD-168,077 did not alter the basal or AII-stimulated phosphorylation of PKC-α/βII Thr638/641, PKC-μ Ser744/748, or PKC-μ Ser744/748 but significantly enhanced the AII-stimulated phosphorylation of PKC-ε Ser729. L-745,870 reversed this augmenting effect of PD-168,077 (Fig. 3, A and B).

Immunoblotting with antibodies for total forms of PKC-α, PKC-βII, PKC-ε, and PKC-μ of H295R cells showed a predominant cytosol fraction of these PKC isoforms. AII significantly translocated these PKC isoforms to the membrane with a reciprocal decrease of their cytosol distributions (Fig. 3C). PD-168,077 enhanced the AII-induced translocation of PKC-ε to the membrane and exerted no effect on other PKC isoforms. The enhancing effect was again reversed by L-745,870. Therefore, D4R selectively augmented AII-stimulated PKC-ε activation. PKC-ε-specific peptide inhibited AII-induced aldosterone secretion and CYP11B2 mRNA expression. Permeabilization of the synthetic PKC-ε-specific inhibitory peptide into H295R cells selectively inhibited the AII-stimulated translocation of PKC-ε to the membrane (Fig. 4A) and reduced the elevations of aldosterone and CYP11B2 mRNA levels by AII (Fig. 4B); a 25% reduction of the aldosterone level was noted. These effects were not observed when the control peptide was induced into the cells or when the cells were treated with the saponin-containing permeabilization buffer only.

D4R and PKC-ε modulated an AII-stimulated [Ca^{2+}]_i increase. The AII-stimulated increase of [Ca^{2+}]_i was augmented by the D4R agonist PD-168,077. PD-168,077 not only augmented the peak [Ca^{2+}]_i but also accelerated the increased rate of [Ca^{2+}]_i after AII stimulation; the plateau of [Ca^{2+}]_i after this initial surge was also augmented significantly (Fig. 5A). The effect of PD-168,077 on AII-stimulated increased of [Ca^{2+}]_i was also observed on the similar experiments with the Ca^{2+}-free medium (Fig. 5B). Delivery of the PKC-ε inhibitory peptide attenuated the AII-stimulated peak [Ca^{2+}]_i, the increased rate of [Ca^{2+}]_i (slope), and the plateau of [Ca^{2+}]_i (Fig. 5C).

D4R modulated the intracellular IP_3 level. A significant elevation of the intracellular IP_3 of H295R cells was noted when AII was added, and a further 25% increase of IP_3 was observed by pretreatment with PD-168,077. The augmenting effect of PD-168,077 was reversed by the D4R antagonist.

Fig. 5. Intracellular Ca^{2+} concentration ([Ca^{2+}]_i) change in H295R cells induced by AII at 10^{-8} mol/l with or without pretreatment of PD at 10^{-6} mol/l for 30 min (A: under standard culture medium; B: under calcium-free culture medium). Peak-baseline, the difference between the peak level after AII and the basal level; slope, the peak-baseline divided by the interval from addition of AII to the time after reaching peak; plateau-baseline, the difference between plateau level 120 s after AII treatment and the basal level. The effect of PD on [Ca^{2+}]_i in terms of peak, slope, and plateau are analyzed. *Significantly different from AII (P < 0.05). C: effect of PKC-ε on AII-stimulated [Ca^{2+}]_i. H295R cells were introduced with either IPep or CPep after perforation by S. The time-course difference of AII + S and AII + S + IPep was demonstrated. †Significantly different from AII + S (P < 0.05; also see the legend to Fig. 4). All experiments were performed in at least triplicate; for each experiment the data for analysis were the mean of 3 measured samples. Values are shown as means ± SD.
Although the role of PKC in regulating CYP11B2 transcriptional activity and aldosterone secretion has been studied in H295R cells (22, 35), the results lacked consensus. An increase of aldosterone secretion was observed when the cells were treated with PKC activator (22, 35), and a PKC inhibitor reduced AII-stimulated aldosterone secretion (4, 14). However, by transfection of H295 cells with constitutively active PKC isoforms, LeHoux and Lefebvre (16) observed that PKC-α, -ε, and -δ inhibited basal and stimulatory hamster CYP11B2 promoter activity, whereas PKC-ζ increased it. Several problems may have contributed to the different results in those experiments. First, the PKC isoforms mediating AII-stimulated CYP11B2 mRNA expression and aldosterone secretion may be species dependent (33). Second, cells subjected to prolonged treatment with phorbol ester or transfected with constitutively active PKC may extensively downregulate the PKC activities and the downstream signaling rather than activate them (27, 34). Finally, although kinaseinactive PKC mutants and PKC regulatory domains have been widely used as dominant-negative inhibitors (33), the high homology among PKC isoforms and the lack of knowledge about the intracellular targets of these mutants make this approach questionable.

H295R cells express various PKC isoforms, whereas AII increased only the activities of PKC-α, -βII, -ε, and -µ but not of PKC-θ, -δ, or -ζ. Among all PKC isoforms that were activated by AII, only the phosphorylation of PKC-ε was altered by D4R. By inducing the PKC-ε-specific inhibitory peptide, we found that the activation of PKC-ε contributed partially, at least 25%, to the aldosterone synthesis/secretion induced by AII. Recently, we (6) found that inhibition of PKC-µ activity by its specific shRNA could reduce AII-stimulated CYP11B2 mRNA level and aldosterone secretion by ~80%. Therefore, activations of both PKC-µ and PKC-ε account for most AII-induced aldosterone synthesis/secretion. In comparison, PKC-µ may be more important in the regulation of aldosterone synthesis/secretion than PKC-ε. This spec-
ulation is supported by our recent data (6) that an increase in PKC-ε phoshorphylation is ubiquitously present in aldosterone-producing adenomas.

The parallel, rather than a reciprocal, change of intracellular and extracellular levels of aldosterone indicates that AII induces acute steroidogenesis instead of an exocytosis of secretory vesicles. This acute steroidogenesis is independent of CYP11B2 transcription and may involve the following mechanisms: 1) transfer of free cholesterol to the outer mitochondrial membrane via a calmodulin-dependent kinase II, 2) activation of the StAR protein by a calmodulin-dependent kinase II-mediated process, and 3) increase in mitochondrial Ca2+ leading to activation of mitochondrial dehydrogenases of the tricarboxylic acid cycle and NAD(P)H production (27). D4R, like D2R, can modulate the [Ca2+]i, induced by AII and, in turn, aldosterone secretion. The modulation of [Ca2+]i by D4R is probably through its effect on intracellular IP3 production.

Stimulation of the AT1R initiates a cascade of signaling events, including the activation of phosphoinositide-specific phospholipase C and the hydrolysis of phosphatidylinositol 4,5-bisphosphate to yield soluble IP3 and diacylglycerol; the latter can activate PKC. We noted that D4R could enhance AII-induced elevation of [Ca2+]i, even when the extracellular calcium was absent. This result suggests that D4R enhances AII-induced [Ca2+]i elevation via increasing [IP3]i, level, which through IP3 receptors releases calcium from the intracellular calcium stores. Interestingly, inhibition of PKC-ε activity can attenuate AII-induced elevation of intracellular IP3 and [Ca2+]i. Therefore, the effect of D4R on [Ca2+]i elevation can also be mediated by activation of PKC-ε, which is calcium independent (33). However, the augmentation of [Ca2+]i by D4R occurred much earlier (within seconds) than its effect on AII-induced PKC-ε activation (within minutes; data not shown). Therefore, the augmentation of AII-stimulated elevation of [Ca2+]i by D4R may be biphasic.

How can D4R enhance the immediate AII-stimulated [Ca2+]i increase? D2R has been reported (24) to have a direct negative coupling with phospholipase C (PLC) by heterotrimeric G1,2,3 proteins in rat anterior pituitary cell membrane. Dopamine and D2 agonists inhibited TRH- and AII-stimulated membrane PLC activities (11a). Our recent report has shown that the D2 agonist bromocriptine can attenuate AII-stimulated intracellular IP3 accumulation, a finding that has also suggested that there is negative regulation of D2R on the PLC activity. In contrast to D2R, we (11) found that D4R enhanced AII-stimulated intracellular IP3 accumulation. D4R has been proven to stimulate PLC activity in prefrontal cortex. It is possible that D4R could be able to enhance PLC activity through its coupling G protein components. By enhancing AII-stimulated PLC activity, D4R could increase AII-stimulated IP3 accumulation and thereafter Ca2+ release from intracellular stores through IP3 receptors.

Our experiments showed that the D4R-modulated effects on aldosterone secretion/synthesis occurred only in the presence of AII. It is also possible that the effects are mediated through a direct interaction of these two G protein-coupled receptors to enhance the downstream signaling (1). D4R has been reported (30) to mediate inhibition of potassium current in neurophysial nerve terminals. D4R could also inhibit the potassium current of H295R cells and partially depolarize the membrane potential that enhances AII-induced T-type calcium channel opening. D2R may regulate the “negative regulating proteins” such as phosphatases through spinophilin, a protein phosphatase-1-interacting protein (26). It is also possible that D4 inhibits the phosphatase that plays role in turning down the activated PKC-ε from the AT1R. In addition, we found that inhibiting activation of PKC-ε attenuated both AII-induced intracellular free calcium increase and IP3 accumulation. Although this mechanism is still not clear, it is possible that PKC-ε enhanced PLC ACTIVity in the adrenal cortical cells. By way of inhibition of PLC activity, PKC-ε inhibitory peptide attenuated AII-induced IP3 accumulation and intracellular free calcium increase. We illustrate the possible signals of D4R-modulated effects in Fig. 7.

In summary, similarly to some other families of G protein-coupled receptors, among D2-like receptors, D4R has opposing effects to D2R in AII-stimulated aldosterone secretion. D4R not only upregulates CYP11B2 expression but also enhances acute aldosterone synthesis. The modulation is mediated through enhancing of PKC-ε and of [Ca2+]i, elevation.

ACKNOWLEDGMENTS

We thank the staffs of the 2nd Core Laboratory, Department of Medical Research, National Taiwan University Hospital, for technical support.

GRANTS

This work was supported by National Science Council Grants NSC-91-2314-B-002-340, 92-2314-B-002-190, and NSC93-2314-B-002-141 (to K.-D. Wu) and the Hsu-Chin Lee Kidney Research Fund.

REFERENCES