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1,25(OH)$_2$-Vitamin D$_3$ Affects the Subcellular Distribution of Protein Kinase C Isoenzymes in Rat Duodenum: Influence of Aging

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Abstract We have previously shown that the steroid hormone 1,25-dihydroxy-vitamin D$_3$ [1,25(OH)$_2$D$_3$] stimulates total cell protein kinase C (PKC) activity in rat duodenum, an effect that is severely impaired in old animals. We further examined the role of 1,25(OH)$_2$D$_3$ on PKC as it relates to aging by measuring hormone-induced changes in subcellular localization of PKC activity and isoenzymes in duodenal mucosae from young (three-month-old) and aged (24-month-old) rats. Short treatment of duodenum with 1,25(OH)$_2$D$_3$ (0.1 nM, 1 min) increased membrane-associated PKC activity, whereas it decreased the activity in the cytosol of young rats but was without significant effect in aged animals. Furthermore, the ability to translocate was present in young animals after a short treatment with the phorbol ester 12-O-tetradecanoyl phorbol 13-acetate (TPA; 100 nM) or dioctanoyl-glycerol (50 μM), whereas the ability was absent in aged rats, suggesting that PKC function was impaired with aging independent of agonist stimulation. The expression of specific PKC isoenzymes and changes in their subcellular distribution after short exposure of the duodenum to the hormone were determined. Western blot analysis of total homogenates using antibodies to various PKC isoforms allowed detection of PKC α, β, and δ. The expression of the θ and the ζ isoforms was in addition demonstrated by reverse transcription-polymerase chain reaction. The pattern of isoenzymes present in the duodenum was unaffected by aging. In young rats, 1,25(OH)$_2$D$_3$ translocates PKC α, β, and δ to the membrane and nucleus; however, no translocation of PKC isoforms was observed in 24-month-old animals in response to the hormone. In summary, in rat duodenum, 1,25(OH)$_2$D$_3$ modulation of PKC activity and isoenzyme subcellular distribution are impaired with aging and may explain age-induced alterations in the intestinal processes under the control of the hormone. J. Cell. Biochem. 79:686–694, 2000.

Key words: rat duodenum; 1,25(OH)$_2$-vitamin D$_3$; protein kinase C; PKC isoenzymes; aging

The steroid hormone 1,25-dihydroxy-vitamin D$_3$ (1,25(OH)$_2$D$_3$), the major biologically active form of vitamin D$_3$, plays an essential role in the regulation of calcium and phosphate homeostasis [De Luca et al., 1990]. In addition to this classic function, 1,25(OH)$_2$D$_3$ has been involved in several physiologic responses unrelated to mineral homeostasis, such as regulation of cell proliferation and differentiation of several cell types [Walters, 1992]. The effects of the hormone are mediated through a specific vitamin D nuclear receptor that modulates gene transcription [Minghetti and Norman, 1988] and by a genomic-independent mechanism that involves activation of signal transduction pathways [de Boland and Nemere, 1992; de Boland and Boland, 1994]. In several tissues, 1,25(OH)$_2$D$_3$ rapidly stimulates calcium influx and the turnover of phosphoinositides, leading to increases in the levels of inositol trisphosphate (IP$_3$), which releases Ca^{2+} from internal stores, and diacylglycerol (DAG), the natural signal for protein kinase C (PKC) activation [de Boland et al., 1994; Morelli et al., 1993].

PKC, a family of phospholipid-dependent serine-threonine kinases, plays a key role in the regulation of cell growth and differentiation and modulation of ion channels [Nishi-

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zuka, 1992]. Although PKC isoforms have many structurally similar elements, they differ from one another in cellular and tissue distribution [Wetsel et al., 1992]. While the conventional PKCs (PKC α, βI, βII and γ) require Ca^{2+} for their activation, the novel PKCs (PKC δεη and θ) lack the calcium-binding domain and can be activated without Ca^{2+} [Schaap et al., 1989; Osada et al., 1990; Johannes et al., 1994]. All these isoforms can be activated by phorbol esters in the absence of Ca^{2+}, phospholipids, or DAG. A third group, the atypical PKCs (ζιλ, and μ), require only phosphatidylserine for activation and do not bind to phorbol esters.

The various isoforms are present in soluble and membrane cellular compartments in variable ratios, and enzyme activation is often accompanied by translocation of the kinase from the cytosol to the plasma membrane and nuclear compartments [Zidovetzki and Lester, 1992].

PKC is a key regulatory enzyme in the mechanism of action of 1,25(OH)$_2$D$_3$. Previous studies have demonstrated that PKC activity is acutely augmented in the particulate fraction of muscle cells in response to short treatment with the hormone [Morelli et al., 1993; Marinissen et al., 1994]. Furthermore, in these cells, 1,25(OH)$_2$D$_3$ was shown to modulate voltage-dependent Ca^{2+}-channel activity via PKC [Vazquez and de Boland, 1995]. Fast effects of 1,25(OH)$_2$D$_3$ on PKC have been previously observed in both rat colonic epithelium [Wali et al., 1990] and Caco-2 cells, a human colon cancer-derived cell line [Bissonnette et al., 1994]. In addition, direct activation of PKC α, β, and ε by the hormone has been demonstrated in vitro [Slater et al., 1995]. In rat duodenum, recent work has demonstrated age-related impairment of phospholipase C stimulation by 1,25(OH)$_2$D$_3$, production of the second messengers IP$_3$ and DAG [de Boland et al., 1996], and altered hormone regulation of Ca^{2+} transport through the PKC messenger system [Balogh et al., 1997].

To further explore these rapid biochemical actions of 1,25(OH)$_2$D$_3$ and their relationship to the aging process, we examined hormone-induced changes in subcellular localization of PKC activity and isoenzymes in duodenal mucosae from young (three-month-old) and aged (24-month-old) rats.

MATERIALS AND METHODS

Materials

Hoffmann-La Roche (Nutley, NJ) provided 1,25(OH)$_2$D$_3$. Sigma Chemical Co. (St. Louis, MO) supplied 12-O-tetradecanoyl phorbol 13-acetate (TPA), 1,2-dioctanoyl-glycerol (DOG), leupeptin, aprotinin, Immobilon P (polyvinylidene difluoride, PVDF) membranes, and specific peptide substrate for PKC. [γ-^{32}P]ATP was provided by New England Nuclear (Chicago, IL). PKC antibodies were from Gibco-BRL (Gaithesburg, MD). Oligonucleotides specific for PKC αδεζ, andθ and other reagents for molecular biology were kindly provided by Dr. Primitivo Barja Francisco (Molecular Biology Laboratory, University of Santiago de Compostela, Spain).

Animals

Young (three months) and senescent (22–24 months) Wistar rats were fed with standard rat food (1.2% Ca, 1.0% phosphorus), given water ad libitum, and maintained on a 12-h light/12-h dark cycle. Animals were killed by cervical dislocation. The abdomen was opened via a midline incision, and the duodenum (10 cm from the pilorus) was exposed, everted, excised, and placed on cold physiologic saline.

Treatments

The duodenum was preincubated for 15 min at 37°C in a medium containing 145 mM NaCl, 5 mM KCl, 10 mM Na/MOPS pH 7.3, 5.6 mM glucose, 0.5% bovine serum albumin, 1mM $CaCl_2$, and 1mM $MgCl_2$. The tissue was then incubated in the absence and presence of 1,25(OH)$_2$D$_3$ (0.1 nM), TPA (100 nM), DOG (50 μM), or vehicle ethanol (<0.01%) for 1–5 min as indicated in each experiment. After treatment, the duodenum was rapidly transferred to a glass plate with ice underneath, the mucosa was immediately scraped, and it was homogenized fractionated subcellularly in the cold, as described below. When the treatment with 1,25(OH)$_2$D$_3$, TPA, or DOG was also performed at low temperature, neither agent exerted any effect.

Cellular Fractionation

Subcelullar fractions were isolated from intestinal mucosae as previously described [Balogh et al., 1997]. Briefly, duodenal muco-

sae was scraped and homogenized for 40 s with an Ultraturrax (Jank and Kunkel, Staufen, FRG) homogenizer using an ice-cold buffer composed of 20 mM Tris-HCl pH 7.4, 0.33 M sucrose, 1mM EGTA, 0.7 mM $CaCl_2$, 20 mM NaF, 0.5 nM PMSF, 1 mM dithiothreitol, 40 μg/ml leupeptin, and aprotinin (homogenization buffer). Subcellular fractions were obtained by differential centrifugation at 2°C. The homogenate was centrifuged for 10 min at 500*g* in a Sorvall refrigerated centrifuge. The pellet (nuclear fraction) was resuspended in the same buffer containing 1% Triton X-100 and used as the nuclear fraction. The supernatant was then centrifuged at 100,000*g* for 1 h. The pellet was used as membrane fraction and the supernatant as cytosol. The fractions were resuspended in homogenization buffer containing 1% Triton X-100, and the protein content was measured according to Lowry (Lowry et al., 1951). Measurements of PKC activity and Western Blot analysis were then carried out.

Protein Kinase C Activity

PKC activity was determined in subcellular fractions from duodenal mucosae by measuring the incorporation of ^{32}P from [γ-^{32}P]ATP into the threonine group of a specific sustrate (PRO-LEU-SER-ARG-THR-LEU-SER-VAL-ALA-ALA-LYS-LYS) for PKC [Thomas et al., 1987]. PKC activity was assayed in 80 μl of a solution containing 50 mM Tris-HCl pH 7.5, 15 mM $MgCl_2$, 1 mM EGTA, 12.5 mM NaF, 50 μg/ml leupeptin, 0.2 mM PMSF, and 75 μM binding peptide in the presence or absence of 1 mM $CaCl_2$ and 38 μg/ml phosphatidylserine. The reaction was started by adding 20 μM [γ-^{32}P]ATP. The mixture was then incubated at 30°C for 5 min. The reaction was terminated by transferring radioactive material to an ion-exchange-chromatography paper (Whatman P-81), and washing three times in 30% (v/v) acetic acid for 10 min. The paper was dried, immersed in Aquasol, and the bound radioactivity was measured in a scintillation counter.

Western Blot

Proteins in subcellular fractions and the total homogenate were resolved by one-dimensional sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) [Laemmli, 1970]. Briefly, samples were mixed with 2× Laemmli sample buffer (250 mM Tris-HCl pH 6.8, 8% SDS, 40% glycerol, 20% 2-mercaptoethanol, and 0.02% bromophenol blue) and heated for 5 min at 95°C. Proteins (100 μg) were subjected to electrophoresis on 10% SDS-polyacrylamide gels and then transferred to Immobilon P (PVDF) membranes. The membranes were allowed to dry at room temperature and afterwards immersed in TBS (20 mM Tris-HCl pH 7.5 and 150 mM NaCl) containing 5% skim milk for 2 h to block nonspecific binding. Membranes were then washed (5 min) twice with TBS-T (20 mM Tris-HCl pH 7.5, 150 mM NaCl, and 0.05% Tween 20), followed by one 10-min wash with TBS-T. Anti-PKC isoform antibodies were diluted to 1 μg/ml in TBS-T and were allowed to react with the membrane overnight at room temperature. After washing with TBS-T, membranes were incubated with 1μg/ml of peroxidase-labeled goat anti-rabbit IgG antibody (dilution 1:100) or goat anti-mouse IgG antibody (dilution 1: 500) in TBS-T for 1 h at room temperature. After two washes with TBS-T, the membrane was visualized by using an enhanced chemiluminiscent technique (Amersham Corp.), according to the manufacturer's instructions. Competition assays were carried out employing peptides corresponding to the same regions used to prepare the antibodies. Images from PVDF membranes were obtained with a Hewlett Packard IIp scanner at 300 dpi and printed at the same resolution. The relative amounts of PKC isoenzymes were compared by the Band Leader Program written by Ma'ayan Aharaoni, copyright 1993, taken from Internet.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Total cellular RNA from duodenal mucosae was isolated using the UltraspecTM RNA isolation system (Biotech Lab Inc.) with the guanidinium/phenol-cloroform reagent. cDNA was synthesized in a 20-μl reaction volume containing 1 μg of total cellular RNA, 0.5 μg oligo (dT; Promega), 10 mM DTT, 0.5 mM dNTP (Promega), 50 mM Tris-HCl pH 8.3, 75 mM KCl, 3 mM $MgCl_2$, and 200 units of AMV reverse transcriptase (Gibco BRL). After 60 min incubation at 37°C, the samples were heated at 95°C for 5 min and then quick-chilled on ice. Synthesized cDNA was diluted in PCR buffer (20 mM Tris-HCL pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$). PCR was essentially performed as previously described [Saiki et al., 1988]. PKC isoform-specific primers based on

TABLE I. Primers Used for PKC Isoform-Specific PCR[a]

Oligonucleotide fragments size (bases)	Sequences
PKC α	
PKC-5′	TGAACCCTCAGTGGAATGAGT
324	
PKC-3′	GGCTGCTTCCTGTCTTCTGAA
PKC δ	
PKC-5′	CACCATCTTCCAGAAAGAACG
351	
PKC-3′	CTTGCCATAGGTCCCGTTGTTG
PKC ϵ	
PKC-5′	CATCGATCTCTCGGGATCATCG
731	
PKC-3′	CGGTTGTCAAATGACAAGGCC
PKC ζ	
PKC-5′	CGATGGGGTGGATGGGATCAAAA
680	
PKC-3′	GTATTCATGTCAGGGTTGTCTG
PKC θ	
PKC-5′	CGATGGGGTGGATGGGATCAAAA
734	
PKC-3′	GTATTCATGTCAGGGTTGTCTG

[a]PKC, protein kinase C; PCR, polymerase chain reaction.

reported sequences from the Gen Bank were designed to select unique regions of each isoform for amplification using Mac Vector 4.11 (IBI, New Haven, CT). Expected product sizes are given in Table I. cDNA (1 ng) was mixed with 0.2 mM dNTP, 0.5 μM of each of the 5′ and 3′ primers, 1.5 mM $MgCl_2$, and 2.5 units Taq DNA polimerase (Promega) in 10 μl PCR buffer. The mixture was overlaid with mineral oil and then amplified using a Perkin Elmer/Cetus thermal cycler. An amplification program of denaturation (94°C, 60 s), primer annealing (57°C, 60 s), and primer extension (72°C, 75 s) was employed by repeated cycling with cDNA template. In all experiments, the PCR reaction components were tested for possible contaminants with a 35-cycle reaction with the cDNA template being omitted. PCR products were analyzed by electrophoresis on agarose gels and DNA was visualized by ethydium bromide staining.

Statistical Evaluation

The significance of the results was evaluated by Student's *t*-test [Snedecor and Cochran, 1967].

RESULTS

In agreement with our previous observations [Balogh et al., 1997], 1,25(OH)$_2$D$_3$, at physiological concentrations (0.1 nM), rapidly (1 min) increased (+60%, $P < 0.025$) PKC activity in young rat duodenal mucosae (Fig. 1). The PKC activator DOG augmented intestinal enzyme activity to the same extent as the hormone. With aging, PKC activity is not significantly modified by 1,25(OH)$_2$D$_3$ or DOG. We then examined the effects of the hormone on the subcellular distribution of duodenal PKC activity in young and aged rats. To that end, the duodena from both three-month- and 24-month-old animals were briefly (1–5 min) exposed to 1,25(OH)$_2$D$_3$ followed by isolation of subcellular fractions and measurement of PKC activity. Fig. 2 shows that in young animals, 1-min treatment with 0.1 nM 1,25(OH)$_2$D$_3$ markedly increased PKC activity associated with membranes and to a lesser extent that of the nuclear fraction, whereas it decreased the activity present in the cytosol. The changes in PKC activity for all the subcellular fractions assessed totaled a 70% increase, which is in agreement with the data of Figure 1. The response was transient, reaching values near

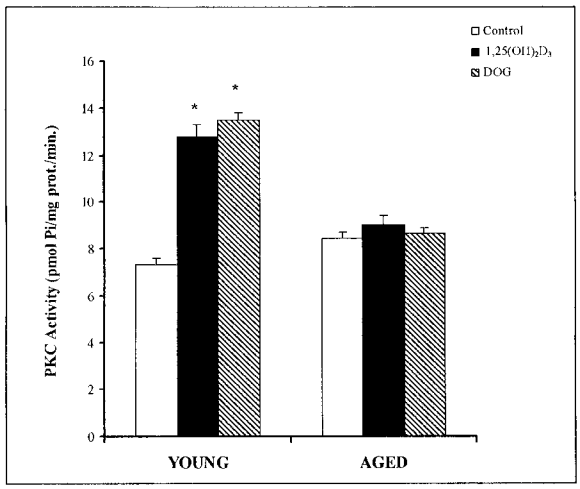

Fig. 1. Effects of $1,25(OH)_2$-vitamin D_3 and 1,2-dioctanoylglycerol (DOG) on rat duodenum protein kinase C (PKC) activity. Duodena from young (three months) and aged (24 months) rats were incubated for 1 min in the absence (control) and presence of 0.1 nM $1,25(OH)_2D_3$ or 50 μ M DOG. The mucosae was scraped and PKC activity was determined by measuring the incorporation of ^{32}P from [$\gamma^{32}P$]ATP into a specific peptide substrate as described in Materials and Methods. Results are the means ± SD of three separate experiments performed in quadruplicate. *$P < 0.025$, with respect to the corresponding control.

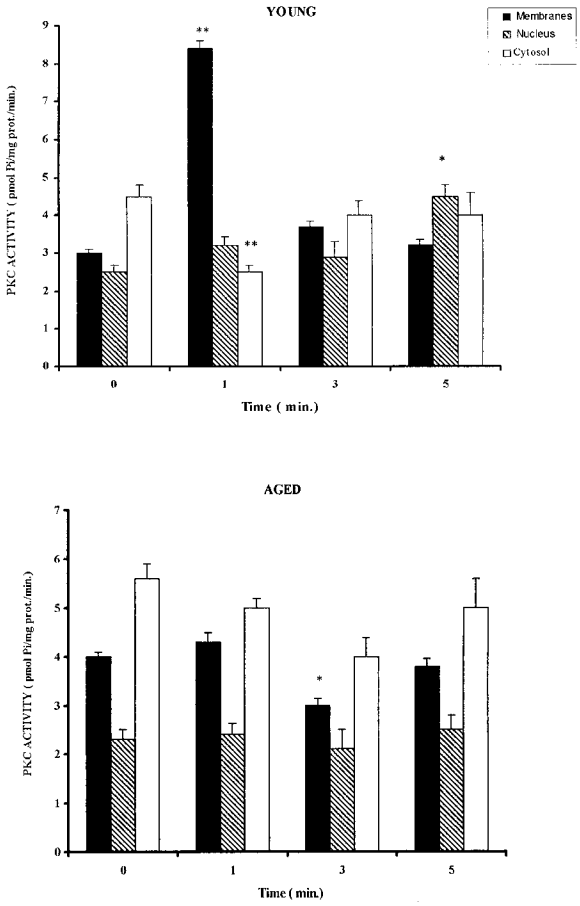

Fig. 2. $1,25(OH)_2$-vitamin D_3 induces protein kinase C (PKC) translocation in rat duodenal mucosae. Duodena from young (three months) and aged (24 months) rats were incubated with 0.1 nM $1,25(OH)_2D_3$ for 1, 3, and 5 min. The mucosae were scraped and subcellular fractions were obtained by differential centrifugation. PKC activity was measured as described in the legend of Figure 1. Results are the means ± SD of six separate experiments performed in quadruplicate. *$P < 0.005$; **$P < 0.001$ with respect to zero time; treatment of duodena with vehicle alone (ethanol, <0.01%) for 1–5 min did not modify the distribution of PKC activity between membranes, nucleus, and cytosol.

basal after 3 min of hormonal exposure. At a longer treatment interval (5 min), an elevation in nuclear PKC activity was also observed (+78%, $P < 0.005$). Incubation of duodena with vehicle alone (ethanol, <0.01%) for 1–5 min did not modify the subcellular distribution of PKC activity. In aged duodenum, $1,25(OH)_2D_3$ was without appreciable effects on PKC activity of different cell compartments at all times analyzed.

In young animals, 100 nM TPA, like $1,25(OH)_2D_3$, significantly increased PKC activity associated with the membrane fraction (+73%, $P < 0.001$) while it decreased that in the cytosol (−24%, $P < 0.001$). No changes were detected in the nuclear fraction. On the other hand, treatment with 50 μM DOG decreased PKC activity associated with the nucleus (−40%, $P < 0.001$), whereas it increased that in the cytosol (+25%, $P < 0.05$). Differential effects of the phorbol ester and the synthetic diacylglycerol on subcellular distribution of PKC activity have been previously observed in other cell types [Marinissen et al., 1998]. In aged animals, neither TPA nor DOG had significant effects on enzyme activity in all the subcellular fractions analyzed (Table II).

The presence of PKC α, β, δ, ζ, ϵ, and θ isoforms in rat duodenal mucosae was investigated by Western blot analysis. Using specific antibodies against these isozymes, only immunoreactive single bands (80 kDa) corresponding to the conventional PKCs α and β and the novel PKC δ were detected (Fig. 3), similar to cytosol from rat brain, which was used as a positive control. In both tissues, PKC β and δ were the predominant isoforms present. No other immunoreactive bands were found when large amounts of duodenal mucosae protein (100–150 μg) were loaded on the gel. The spec-

TABLE II. Effect of Aging on TPA and DOG-Induced Duodenal PKC Translocation[a]

Young	PKC Activity (pmol Pi/mg prot/min)		
Fractions	Control	TPA	DOG
Membrane	3.0 ± 0.08	5.8 ± 0.13**	3.9 ± 0.12
Nucleus	1.9 ± 0.07	2.2 ± 0.09	1.14 ± 0.02**
Cytosol	4.0 ± 0.12	3.1 ± 0.10**	5.0 ± 0.20*
Aged	PKC Activity (pmol Pi/mg prot/min.)		
Fractions	Control	TPA	DOG
Membrane	3.9 ± 0.18	3.8 ± 0.23	4.0 ± 0.32
Nucleus	2.2 ± 0.14	2.1 ± 0.19	2.0 ± 0.22
Cytosol	5.0 ± 0.32	5.3 ± 0.30	5.7 ± 0.15

[a]Duodena from young (three months) and aged (24 months) rats were incubated for 1 min in the absence (control) or presence of 12-O-tetradecanoyl phorbol 13-acetate (TPA; 100 nM) or 1,2-dioctanoyl-glycerol (DOG; 50 μM). The mucosae were scraped and subcellular fractions were isolated by differential centrifugation. Protein kinase C (PKC) activity was measured as described in the legend to Fig. 1. Results are the average ± SD of five independent experiments.
*$P < 0.05$.
**$P < 0.001$.

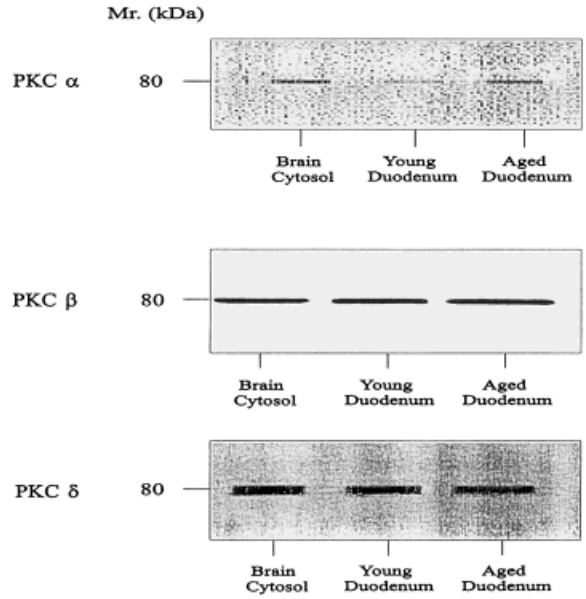

Fig. 3. Expression of protein kinase C (PKC) α, β and δ isoforms in rat duodenum. Duodenal mucosae from young (three months) and aged (24 months) rats were homogenized and the equivalent to 50 μg of protein was electrophoresed, electroblotted to polyvinylidene difluoride membranes and incubated with specific antisera as indicated in Materials and Methods. A Western blot representative of three separate experiments is shown. Rat brain was used as positive control.

ificity of the antibodies was confirmed by competitively abolishing the bands with an excess of the corresponding cold peptide, as well as by the use of rat brain extracts (not shown). Pertinent to the aim of this study, aging did not cause major modifications in the protein levels of duodenal PKC α, β, and δ (Fig. 3). RT-PCR was employed to further investigate the expression of PKC isoforms at the mRNA level. After cDNA synthesis, specific primers were used to amplify PKC isoforms α, δ, θ, ζ, and ϵ. PKCs α and δ, expressed at low and high protein levels, respectively (Fig. 3), were considered positive controls; the β isoform expressed to the same extent as PKC δ was not amplified. Figure 4 shows the agarose gel electrophoresis patterns of the amplified products obtained for young and aged rat duodenum. Clear bands containing fragments of the expected sizes for PKC α, δ, θ and ζ were seen, while PKC ϵ was undetectable. A similar PCR profile was observed in duodenal mucosae from old rats.

The effects of 1,25(OH)$_2$D$_3$ on the distribution of PKC isoforms between the cytosolic, membrane, and nuclear fractions of duodenal mucosae from young (three months) and aged (24 months) rats were examined. As shown Fig. 5, in young duodenal mucosae treated with 1,25(OH)$_2$D$_3$ (0.1 nM, 1 min), PKC δ was translocated to the nucleus and to a lesser extent to the membrane, whereas PKC α and β migrated equally to both the nucleus and membranes. In aged duodenum, however, 1,25(OH)$_2$D$_3$ did not significantly affect the subcellular distribution of PKC isozymes.

DISCUSSION

In this work, evidence was obtained indicating that the steroid hormone 1,25(OH)$_2$D$_3$ at physi-

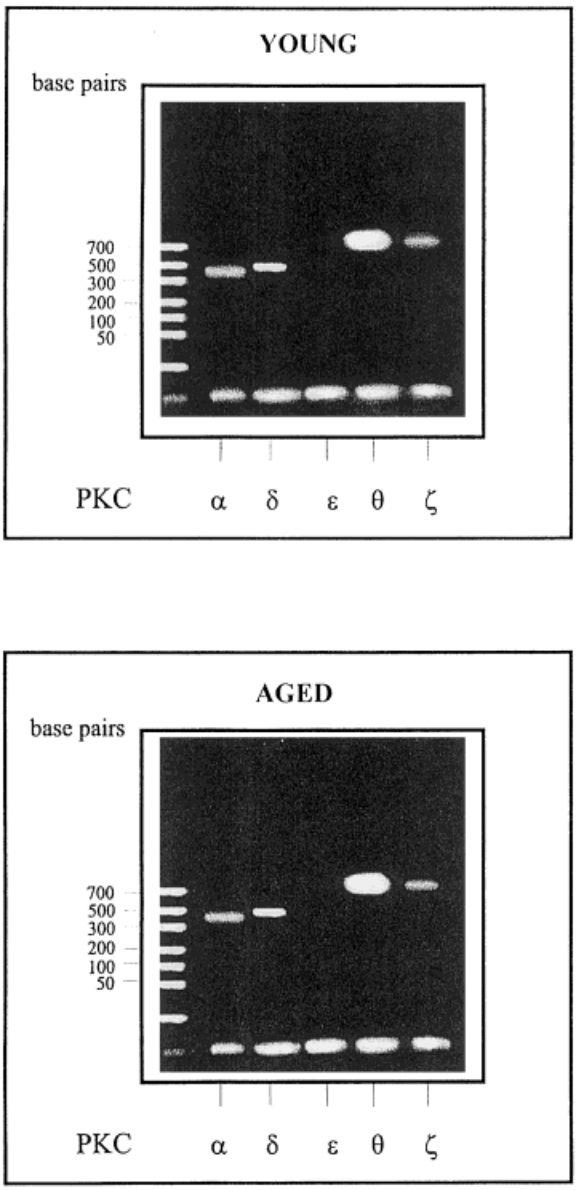

Fig. 4. Agarose gel analysis of DNA fragments generated by reverse transcription-polymerase chain reaction (RT-PCR). Rat duodenum mRNA was reverse transcribed into cDNA and amplified by PCR using protein kinase C (PKC) isoform-specific primers. DNA generated was analyzed on agarose gels and stained with ethydium bromide as described in Materials and Methods. The left lane is a DNA size marker with the base pairs indicated. The expected fragment sizes are shown in Table I. RT-PCR was representative of four independent experiments.

ologic concentrations, rapidly (within 1 min) induces membrane translocation and stimulates PKC activity in rat duodenal mucosae, an effect that is severely impaired with aging. The ineffectiveness of $1,25(OH)_2D_3$ to translocate and activate PKC in old rats is consistent with recent investigations of our laboratory showing age-related impairment of duodenum phospholipase C stimulation by the hormone and the production of the second messengers IP_3 and DAG, the endogenous activator of PKC [de Boland et al., 1996]. However, the ability of PKC to translocate was present in young rats after TPA or DOG treatment, but it was absent in aged rats. This observation indicates that PKC function is also impaired with the aging process independent of agonist stimulation.

Our study provides the first immunologic evidence on the expression at the protein level of the Ca^{2+}-dependent PKC α and β and Ca^{2+}-independent PKC δ isoforms in rat duodenal mucosae. Western Blot analysis demonstrated the presence of specific immunoreactive bands corresponding to macromolecules of 80 kDa. This molecular mass is in agreement with that established for the intact PKC molecule in mammals. The identity of each isoform was confirmed by deletion of the immunoreactive band upon incubation with the appropriate inhibitory peptide. The presence of PKC α, β, and δ subspecies has been previously shown in basal granulated cells from rat duodenal mucosae by immunohistochemistry, electron microscopy, and confocal laser scanning microscopy [Kawakita et al., 1995]. In human and rat colonic mucosae, the expression of both calcium-dependent (classic) and calcium-independent (novel and atypical) PKCs have been demonstrated [Davidson et al., 1994]. Although PKCs ζ, ϵ, and θ were not detectable by immunoblotting in rat duodenal mucosae, we showed the presence of mRNA for these isoforms, while PKC ϵ was not detected. Protein kinase C isoenzymes are distributed between the cytosolic and particulate fractions depending on cell type and growth characteristics. Upon cell activation by agonists, the soluble forms of PKC usually translocate to the membrane [Nishizuka, 1992]. In nonstimulated duodenum, we found that PKC α and β were equally localized in the particulate and cytosolic fractions, while PKC δ amounts were somewhat higher in cytosol. In young duodenum treated with $1,25(OH)_2D_3$, we observed that PKC α, β, and δ isoforms translocate to both nuclear and membrane fractions, whereas the hormone translocation of PKC isoforms was not observed in 24-month-old animals.

In view of the cellular roles of PKC, the results of this study may be significant for understand-

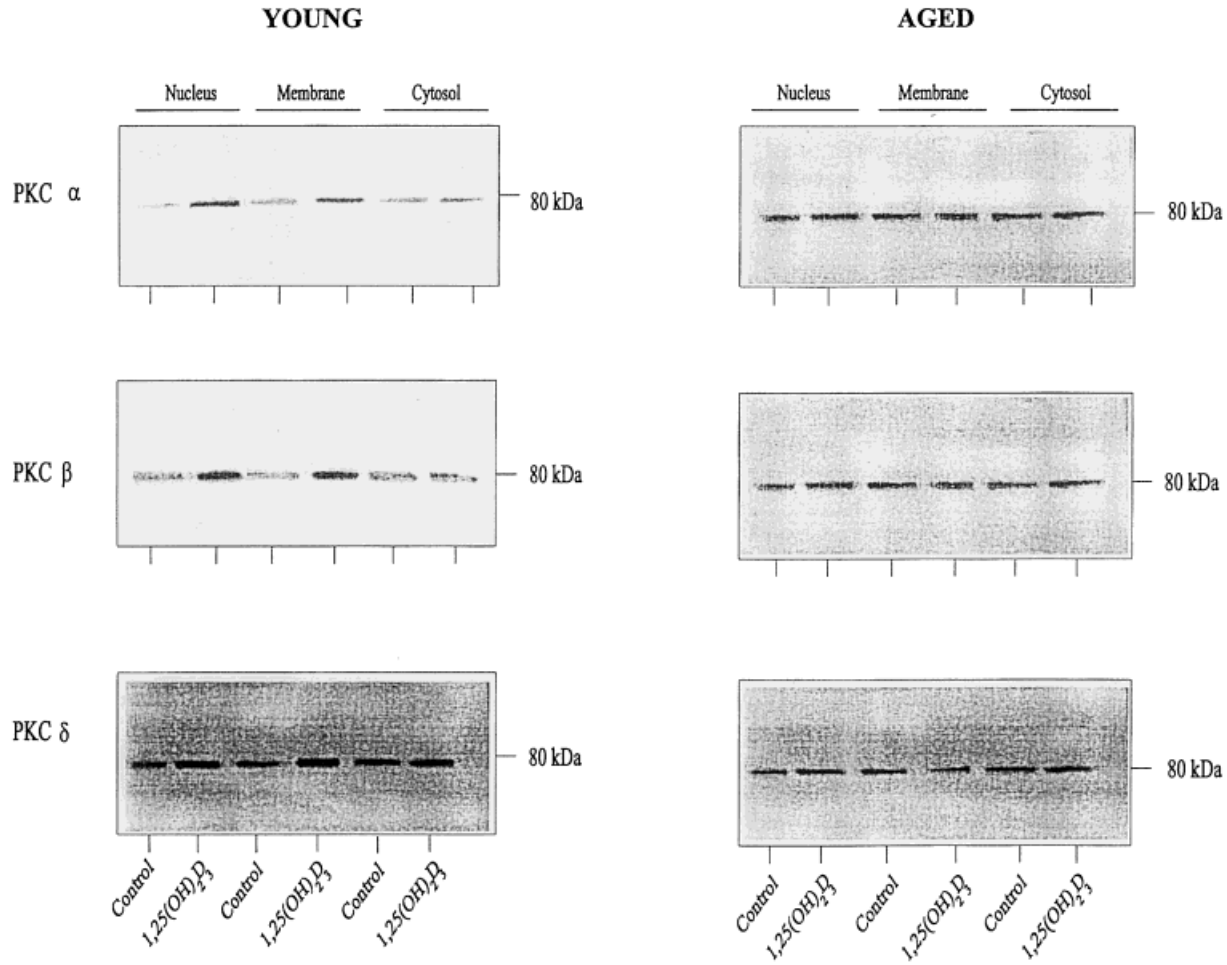

Fig. 5. Effects of 1,25(OH)$_2$-vitamin D$_3$ on the subcellular distribution of protein kinase C (PKC) α, β, and δ isoenzymes in duodenal mucosae from young and old rats. Duodena from young (three months) and aged (24 months) rats were treated for 1 min with 0.1 nM 1,25(OH)$_2$D$_3$ or vehicle ethanol (control). The mucosae were scraped and homogenized; the cytosol, nuclear, and membrane fractions were obtained. Immunoblot analysis was performed using specific antibodies against PKCs α, β, and δ isoforms as described in the legend of Figure 3. A Western blot representative of three separate experiments is shown.

ing the mechanism of action of 1,25(OH)$_2$D$_3$ in the intestine and the alterations that ensue upon aging. Phosphorylation by PKC influences long-term events such as proliferation and differentiation by stimulating cascades that target the nucleus, as well as by translocation of the enzyme itself to the nucleus. In addition, PKC modulates ion channel activity [Nishizuka, 1992]. In relation to the latter role, there is evidence indicating that PKC mediates 1,25(OH)$_2$D$_3$ regulation of Ca^{2+} transport across the duodenum [de Boland and Norman, 1990; Balogh et al., 1997]. The vitamin D endocrine system is involved in the reduction of intestinal calcium absorption observed with aging [Armbrecht et al., 1989], which has been related to an impairment in both the genomic and the nongenomic modes of action of 1,25(OH)$_2$D$_3$ [Takamoto et al., 1990; Massheimer et al., 1995]. Relevant to this work, recent investigations have shown that 1,25(OH)$_2$D$_3$ regulation of duodenal Ca^{2+} transport through the PKC messenger system is diminished with aging [Balogh et al., 1997]. Considering the observations described above, it is then possible that this alteration is connected to the lack of hormone-induced translocation of PKC α, β, and/or δ.

In summary, in rat duodenum, 1,25(OH)$_2$D$_3$ modulation of PKC activity and isoenzyme subcellular distribution is impaired with aging and may explain age-induced alterations in the intestinal processes under the control of the hormone.

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