Inhibition of Expression of Protein Kinase C α by Antisense cDNA Inhibits Phorbol Ester-mediated Arachidonate Release*

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A major unresolved issue in the area of signal transduction relates to the role of particular isoforms of protein kinase C (PKC) in mediating cellular responses subsequent to activation of that enzyme. We have addressed this issue by the use of antisense technology. We have stably transfected Madin-Darby canine kidney cells with antisense PKCα, PKCβ, or both PKCα and -β cDNAs. The transfected cDNA was integrated and expressed. We have isolated cells in which expression of PKCα is inhibited. In cells transfected with antisense PKCα or both PKCα and -β, phorbol ester-stimulated release of arachidonate and its metabolites was inhibited, whereas in cells transfected with antisense PKCβ cDNA alone, phorbol ester-stimulated arachidonate release was not significantly different from control cells. We thus demonstrate the use of a novel technique to inhibit PKC isoform expression. We show that inhibition of expression of PKCα causes a loss in phospholipase A2-mediated arachidonate release. Antisense-inhibited expression of PKC isoforms may provide a useful approach to define additional functions of particular PKC isoforms.

Protein kinase C (PKC) is a serine-threonine protein kinase that plays a critical role in a plethora of cellular responses (1). The classical model of PKC activation invokes a scenario whereby diacylglycerol produced by agonist-mediated phospholipid hydrolysis activates the enzyme inducing a conformational change, thereby facilitating its binding to phospholipid cofactors and to calcium (2). Tumor-promoting phorbol esters, e.g. phorbol 12-myristate 13-acetate (PMA) and phorbol 12,13-dibutyrate, can substitute for diacylglycerol as activators of the enzyme enhancing the association of the cytosolic enzyme with the membrane (i.e. translocation) (3). Prolonged stimulation with phorbol esters causes "down-regulation" of PKC as the membrane-associated form of the enzyme is more susceptible to proteolysis (4).

PKC is not a single enzyme but a family of distinct proteins encoded by individual genes. The α, β, and γ forms of PKC were the first to be cloned (1) and to be characterized biochemically (5). (PKCβ actually consists of two forms that are alternatively spliced variants of a single gene.) PKCα, -β, and γ are all phospholipid-, diacylglycerol-, and calcium-dependent. However, subtle differences in their cofactor dependences, their susceptibility to down-regulation, and their tissue-specific expression has led to the speculation that different isoforms perform specific functions. The more recently described δ, ε, ξ, η, and ι PKC isoforms are also dependent on phospholipid and diacylglycerol for their activation, but lacking the putative calcium binding site expressed in the regulatory region of PKCα, -β and -γ, they are calcium-independent and show distinct in vitro substrate specificities (6).

It has been difficult to demonstrate specificity of function of a single isoform in vivo. As isoform-specific inhibitors of PKC are not available, typical experimental paradigms used to implicate a particular form of PKC are its translocation and/or phorbol ester-induced down-regulation (e.g. Refs. 7-9). Such approaches provide circumstantial evidence implicating differential activation of PKC isoforms in a specific process. However, the down-regulation paradigm has the complicating factor of "activation" of the kinase being a prerequisite for its down-regulation (4). Additionally, recent evidence suggests that PKC translocation may not be required for its activation (10). Overexpression of PKC isoforms has recently been used to implicate them in particular functions (e.g. Refs. 11 and 12). However, in these systems, substrate availability, ligand receptor number, or other regulators may critically limit responsiveness of the system. In an effort to circumvent these problems, we have developed a strategy to isolate stably transfected cells deficient in a specific isoform in order to determine whether a specific PKC-mediated response is altered.

**EXPERIMENTAL PROCEDURES**

**Materials**

[γ-32P]ATP (3000 Ci/mmol), [α-32P]dCTP (3000 Ci/mmol), [3H] arachidonate (100 Ci/mmol), and [125I] Protein A (8.7 μCi/μg) were purchased from Du Pont-New England Nuclear. Histone H11, diolene (1,2-dioleoyl-sn-glycerol, C18:1, cis-9), and PMA were purchased from Sigma. Phosphatidylserine was obtained from Avanti Polar Lipids Inc. pBKS plasmid was obtained from Stratagene. RcCMV mammalian expression vector containing the neomycin resistance gene was purchased from In Vitrogen, La Jolla, CA. PKCα (AhPKCα-7) and PKCβ (AhPKCβ-115) human cDNA probes (13, 14) were obtained from the American Tissue Culture Collection repository of genes.

**Methods**

**Plasmid Constructs**—All constructions were made according to standard procedures (15). 1.3- and 1.7-kb EcoRI fragments containing PKCα or PKCβ, respectively, were isolated from the pUC host vector and subcloned into the mammalian expression vector pCMV-1 in the...
antisense orientation using Csl and XhoI (PKCa and RefII and Csl (PKCB)). This yielded antisense PKC (PKF0 and PKF9) preceded by the CMV promoter and followed by the human growth hormone termination and polyadenylation signals (Fig. 1). All constructs were confirmed by sequence analysis.

Cells and Transfections—MDCK-D1 cells were cultured as previously described (18). Cells (approximately 1 x 106 cells/100-mm dish) were co-transfected with pCMV expression vectors containing antisense PKCβ and/or PKCδ and the neomycin resistance gene (RC-MVMVNeo, Invitrogen) using calcium phosphate coprecipitation (15). Control cells were transfected with RC-MVMVNeo only. A 5-fold mass excess of PKC cDNA over the selectable plasmid was used in transfections. For post-transfection, the cells were plated in medium containing 800 µg/ml G418 (GIBCO/BRL). Colonies of resistant cells appeared after ~18 days and were picked for expansion at 21 days. Stock cultures of these cells were routinely grown in the presence of selective pressure.

Southern Blot Analysis—Genomic DNA from the transfectants and parental cells was prepared by the method of Herrmann and Frischaufl (17). Genomic DNA (30 µg) was digested with EcoRI, separated on a 1% agarose gel, and, following denaturation and neutralization, was transferred to nitrocellulose. The blots were probed with specific PKCα and PKCδ cDNA probes random hexamer-primed (U.S. Biochemical Corp. random priming kit) to a specific activity ~1 x 106 cpm/µg. Bound probe was detected by autoradiography. Southern analyses were performed twice on each of the various cell lines.

Northern Blot Analysis—Total RNA was isolated from stable transfectants and wild-type MDCK-D1 cells by the method of Chomczynski and Sacchi (18). 30 µg of total RNA from each cell type was electrophoresed on a 1.2% formaldehyde gel and transferred to nitrocellulose. Loading of approximately equivalent quantities of RNA per lane was verified by ethidium bromide staining of the gel. The blots were probed with PKCα and PKCδ random hexamer-primed cDNA probes, as described above. Northern analyses were performed twice on the transfectants.

Western Blot Analysis—Lysates from transfectants and parental cells were prepared as described elsewhere (18) substituting 1% Nonidet P-40 for Triton X-100 in the lysis buffer. Equivalent amounts of protein were resolved by SDS-polyacrylamide gel electrophoresis before transferring to nitrocellulose and probing with PKC isoform-specific antisera (19). Bound antibody was detected using [35S]-Protein A. Western blotting experiments were carried out at least three times on the transfectants.

PKC Activity—PKC activity in lysates from the transfectants and wild-type cells was measured by determining diacylglycerol and phospholipid-dependent histone phosphotransferase activity after partial purification of the kinase by DEAE-Sephacel chromatography (16). Arachidonate-PMA-stimulated release of arachidonic acid and arachidonate metabolites (AA) from wild type and transfectants was measured by a method similar to that previously reported from our laboratory (20). Cells were labeled with [3H]arachidonic acid (0.3 µCi/ml of medium) for 18 h. After gently washing with Dulbecco's modified Eagle's medium, 20 mM Hepes, 0.05% bovine serum albumin (incubation medium), the cells were then treated with various concentrations of PMA (0–1 µM) in incubation medium for 60 min at 37 °C. H+ released into the extracellular medium was determined by liquid scintillation counting.

RESULTS AND DISCUSSION

To date it has been difficult to ascribe specific functions to PKC isoforms with certainty. A novel approach to this end is to selectively inhibit expression of the kinase and then to determine if specific responses are altered. Antisense technology provides a unique approach to this problem. The basis of this technique is that expression of native proteins can be inhibited by hybridization of native mRNA to complementary nucleotide sequences.

In preliminary experiments we treated MDCK cells in culture with high concentrations of oligodeoxynucleotides generated complementary to unique 18-nucleotide sequences in the 5' region of PKC. Although the initial data demonstrating decreased in vitro PKC activity relative to sense-treated controls were promising, attempts to optimize the system by establishing the kinetics of uptake of polynucleotide kinase-labeled oligodeoxynucleotides revealed extensive intracellular digestion of oligodeoxynucleotides within 12 h of addition to the extracellular medium. This approach is probably more appropriate for inhibition of expression of proteins with relatively short half-lives (e.g. Refs. 21 and 22).

Thus, we decided to use an alternative strategy: stable transfection of PKC isoform cDNA. Cells were cotransfected with cytomegalovirus promoter-based expression vectors containing the neomycin resistance gene and either antisense PKCα (pCMVCKPα) or antisense PKCδ (pCMVCKPδ), or both (pCMVCKPα + pCMVCKPδ). Control cells were transfected with the neomycin resistance gene only. Stable transfectants were isolated on the basis of colony formation in 800 µg/ml G418. Having isolated resistant colonies, we then investigated integration and expression of the transfected genes using Southern and Northern analysis, respectively.

Integration of transfected DNA into the host MDCK-D1 genome was assayed by Southern blotting. As shown in Fig. 1, panel A, probing of EcoRI-digested genomic DNA from the wild-type parental and transfected cells with a PKCα cDNA probe revealed hybridization to approximately 7.0- and 5.5-kb fragments in all cells (i.e. wild-type, PKPα, PKPδ, and cells transfected with the neomycin resistance gene alone (neo+)). In cells transfected with the pCMV PKPα construct we detected an additional fragment at 1.3 kb, thus indicating incorporation of PKPα into the genomic DNA of the appropriate transfectants. All the transfectants harboring the PKPα construct appear to do so at approximately the same copy number. Probing EcoRI-digested genomic DNA with a PKPδ probe revealed strong hybridization to a 7.0-kb fragment and much weaker hybridization to 6.5- and 5.8-kb fragments in all cells, whereas in cells transfected with PKPδ an additional signal was detected at 1.7 kb, indicative of the integration of PKPδ into the genomic DNA of the transfectants (Fig. 1, panel B).

Having determined that the transfected genes had been integrated into the host cell genome, we used Northern analysis to assess transcription of the DNA. The PKCα and PKPδ probes hybridized to transcripts of approximately 4.4 kb in all cells (wild type and transfectants). In PKPα- and PKPδ-transfected cells additional transcripts were detected at ~1.9 and 2.3 kb, respectively (Fig. 2, A and B). This corresponds to the anticipated size of transcripts produced by the transfected genes. The size of the major native transcript detected agrees well with that reported by others (10, 14, 15). Densitometric analysis of the Northern blots showed an approximately 5-fold excess of PKCα mRNA over the endogenous PKCα transcript and a 1–3-fold excess of PKPδ mRNA over the native PKPδ message.

Given the demonstrated integration and expression of the transfected genes, we then addressed whether expression of the antisense RNA for the PKC isoforms was capable of inhibiting translation of the proteins. To investigate this we used Western blotting with isoform-specific antisera (19). Fig. 3 shows a representative series of PKCα protein levels in various transfectants. These data demonstrate inhibition of expression of PKCα in PKPδ and in PKPα- + PKPδ-transfected cells. Densitometry of the immunoblots shows a decrease in protein expression to approximately 30% of control in some cell lines (there was considerable variability in levels of expression of the proteins between the various transfectants). The slight decrease in expression of PKCα in PKPδ cells may represent cross-reactivity of the antisera. Unfortunately, despite numerous attempts, we were unable to detect PKPδ in Western blotting experiments (although it is easily detectable by immunocytochemical techniques). Thus we are
Fig. 1. Southern analysis of the transfectants. The schematics in the upper panels represent the CMV-PKCa (panel A) and CMV-PKCβ (panel B) constructs depicting the expression vector containing the CMV promoter, human growth hormone (hGH) termination sequence, and orientation of the PKC genes. 30 µg of EcoRI-digested genomic DNA isolated from wild-type (WT) cells and representative examples of cells cotransfected with antisense PKCβ (CKPβ) or PKCa and β-antisense (CKPα + ß) and from cells transfected with the neomycin resistance gene alone (Neo') as indicated were separated on a 1% agarose gel, transferred to nitrocellulose, and probed with PKCa or PKCβ cDNA probes (panels A and B, respectively) random hexamer-primed to a specific activity >1x10^6 cpm/µg. The blot was prehybridized by incubation at 42°C in 5×SSPE, 5× Denhardt's, 50 µg/ml salmon sperm DNA, 50% formamide for 2-4 h. The blot was incubated overnight with 1×10^6 cpm of probe/ml of hybridization solution at 42°C and then washed to relatively high stringency before autoradiography. The migration of a 1-kb ladder (GIBCO/BRL) is shown at the left side of each blot. Arrowheads indicate the anticipated sizes of the integrated genes. In cells cotransfected with CKPα equivalent hybridization to that shown in lanes 5–10 was observed.

Fig. 2. Northern analysis of the transfectants. 30 µg of total RNA isolated from wild type (WT) cells and representative examples of CKPβ, CKPα + CKPβ, and neo' transfectants as indicated were separated on a 1.2% agarose formaldehyde gel, transferred to nitrocellulose, and hybridized to random hexamer-primed PKCa and PKCβ cDNA probes, as described in the legend to Fig. 1. The blot shown in panel A was probed with PKCa cDNA; the blot shown in panel B was probed with PKCβ cDNA. The migration of an RNA ladder (GIBCO/BRL) is indicated on the left side of the blots. The transcripts detected at approximately 1.9 and 2.3 kb in the PKCa and -β transfectants are the appropriate sizes (1.3 and 1.7 kb, respectively) combined with the 0.6-kb polyadenylation signal from the human growth hormone gene. In cells cotransfected with CKPα alone hybridization was observed at 1.9 kb with the PKCa probe only.}

Fig. 3. Western analysis of the transfectants. 150 µg of cell protein from lysates of wild type, CKPα, CKPβ, CKPα + CKPβ, and neo' transfectants as indicated were separated on 10% SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose. The blot was probed with PKCa-specific antisera and bound antibody detected using 125I-Protein A. Purified PKC was used as a positive control in these experiments, and its migration is indicated at the right-hand side of the blot.

unlable to show definitively that the antisense PKCβ gene, despite its integration and expression (Figs. 1 and 2), inhibited expression of the protein by Western blotting.

Of the stably transfected cell lines established, no false positives were obtained in screening the co-transfectants for integration and expression of the transfected genes (from 20 expanded colonies). However, despite continuous selective pressure on the transfectant cell lines, expression of the antisense genes was substantially reduced with time. This was evident by a considerably weaker signal in Southern and Northern blots relative to the endogenous gene or transcript, respectively. Additionally, decreased expression of the proteins was not detected in Western blots of extracts from stably transfected cells of higher passage number. The data presented in this paper were obtained from transfectants between passages 5 and 20.

In addition to determining expression of the PKC isoforms in Western blots, we measured total PKC activity in cell lysates using histone as a substrate. In all antisense PKC cDNA transfected cells a decrease in PKC activity was observed (Fig. 4). This decrease in enzyme activity showed variability between the cell lines established from transfection with the various cDNA vectors CKPa, CKPb, or CKPa + CKPβ and did not correlate exactly with the relative magnitude of the decrease in protein expression observed in the immunoblotting experiments. A significant (p < 0.005) decrease in histone phosphotransferase activity was observed in CKPα and CKPα + CKPβ transfectants compared with either the wild-type or neo' controls. It is likely that some of the residual PKC activity results from expression of non-calcium-dependent isoforms, in particular PKC, in MDCK cells (23). In the CKPβ transfectants PKC activity was reduced to approximately 70% of the wild type. This relatively modest decrease (insignificant compared with the neo' cell lines) may reflect the relative amount of the α and β present in the cells.

2 In a single transfectant cell line, positive for both integration and expression of CKPa, no decrease in PKCa was observed.
the decreased expression of PKC in the antisense transfecteds is unlikely to explain the CKPa or absence of phosphatidylserine and diacylglycerol. Results shown defined as the difference in phosphorylation of histone in the presence or absence of phosphatidylserine and dicetylphosphate. Results shown are expressed as a percentage of PKC activity in the wild-type cells and are the mean ± S.E. of at least four independent experiments performed in triplicate. Cells were labeled with "H-labeled AA overnight, the labeling medium was removed, and, after washing, cells were incubated with 50 nM PMA for 40 min, and the release of "H-labeled arachidonate and its metabolites into the extracellular medium was determined. Results shown are the mean ± S.E. of four experiments; each experiment was performed in duplicate.

as both have similar $K_m$ values for histone phosphorylation (6). However, the histone phosphotransferase activity in the CKPa+CKPβ transfectants is lower than in the CKPa transfectants alone is unclear. On the basis of decreased specific isofrom expression and decreased PKC activity, CKPa, CKPβ, and CKPa+CKPβ transfectants were selected for further experiments.

There are several possible mechanisms whereby expression of antisense RNA can block expression of a target gene (e.g. Ref. 24). These include accelerated turnover of the sense/antisense duplex, which would decrease abundance of target mRNA, decreased transportation of the sense-antisense duplex from the nucleus, inhibition of mRNA binding to the ribosome with subsequent inhibition of protein translation, and prolongation of ribosomal pausing during translation leading to a reduction in protein translation. In our system accelerated turnover of the duplex is unlikely to account for the decreased expression of PKC in the antisense transfecteds as we do not observe a difference in mRNA for the endogenous PKC genes between the transfecteds and the wild-type cells. Our observation that expression of excess antisense RNA inhibits expression of the protein may be due to any of the alternative potential mechanisms described above or to a combination thereof.

The major objective of this work was to determine whether selective inhibition of a PKC isoform alters a specific biological response: PMA-stimulated release of arachidonate and arachidonate metabolites. Arachidonate formation is the rate-limiting step in the synthesis of several potent biological mediators such as prostaglandins and leukotrienes. Previous work from this laboratory has strongly suggested that AA release in response to phorbol esters and to hormones is due to phospholipase $A_2$ activation in this system (25). In CKPa and CKPa+CKPβ transfectants, we observe negligible AA release in response to PMA, whereas in parental cells and neo' cells a 4-fold increase was observed at 50 nM (Fig. 4). In CKPβ transfectants, a 3.5-fold increase in AA release was observed in response to 50 nM PMA and thus was not significantly different from that of the control cells. The modest decrease in AA release from the CKPβ transfectant may reflect slight inhibition of PKCa expression in these transfecteds due to the high sequence homology between the isoforms; however, as we do not see an additive effect on inhibition of AA in CKPa+CKPβ versus CKPa transfants we discount this possibility. These data substantiate work using translocation and down-regulation paradigms to implicate PKCa, but not PKCβ, in PMA- and hormone-stimulated AA release (13). As a further control for the effects of antisense PKC transfection on AA release, MDCK cells were stably transfected with sense PKCa cDNA engineered so as not to be translated into protein (lacking the carboxy-terminal 56 amino acids). In these experiments PKCa protein expression was unchanged, and PMA-stimulated arachidonate release was similar to that observed in wild-type cells.

A cytosolic PLA2 (cPLA2), which is responsible for hormone-stimulated arachidonate release, has recently been described (26-29). There are several possible mechanisms whereby PKCa might effect activation of cPLA2. These are: regulation of cPLA2-activating proteins such as phospholipase activating protein (30) or lipocortin (31), alterations in transmembrane calcium flux (32), G protein coupling (33), or direct regulation through phosphorylation of the phospholipase (34). We have investigated possible phosphorylation of cPLA2 using PLAA-specific antisera. Immunoprecipitation of cPLA2 from cells labeled with "3P and stimulated with phorbol ester or hormone revealed no detectable difference in phosphorylation of the protein relative to basal conditions (data not shown). In other systems the stoichiometry of cPLA2 phosphorylation and AA release suggest that phosphorylation is a necessary but insufficient stimulus for maximal activation; elevations in intracellular calcium are also required. This is compatible with data showing a synergistic effect of PMA on A23187-mediated AA release (17) and the hypothesis that the ability of an agonist to both activate PKC and to elevate intracellular calcium determines its efficacy in releasing AA (35).

In summary, we have used a novel technique to inhibit expression of a specific PKC isoform. We have shown that inhibition of expression of PKCa causes a loss of PLAA-mediated AA release in response to PMA. The use of antisense to inhibit expression of PKC isoforms may provide a useful approach to study differential activation and function of PKC isoforms.

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REFERENCES

1. Nishizuka, Y. (1986) Nature 313, 661-665
2. Bell, R. M., and Buras, J. (1991) J. Biol. Chem. 266, 4691-4694
3. Krafh, A. S., and Anderson, W. B. (1993) Nature 363, 521-523
4. Young, S., Parker, P., Ulrich, A., and Stabel, E. (1987) Biochem. J. 244, 779-785
5. Huang, R. P., Nakahashi, H., and Huang, P. L. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 8535-8539

FIG. 4. PKC activity assay and PMA-stimulated AA release of the transfecteds. A, histone phosphotransferase activity in detergent-containing lysates of wild-type cells, CKPa, CKPβ, CKPa+CKPβ, and neo' transfecteds was determined. PKC activity was defined as the difference in phosphorylation of histone in the presence or absence of phosphatidylserine and dicetylphosphate. Results shown defined as the difference in phosphorylation of histone in the presence or absence of phosphatidylserine and dicetylphosphate. Results shown are expressed as a percentage of PKC activity in the wild-type cells and are the mean ± S.E. of at least four independent experiments performed in triplicate. B, "H-labeled AA release in response to PMA was determined in wild-type cells and CKPa, CKPβ, CKPa+CKPβ, and neo' transfecteds. Cells were labeled with "H-labeled AA over-night, the labeling medium was removed, and, after washing, cells were incubated with 50 nM PMA for 60 min, and the release of "H-labeled arachidonate and its metabolites into the extracellular medium was determined. Results shown are the mean ± S.E. of four experiments; each experiment was performed in duplicate.
Antisense Inhibition of Protein Kinase C

6. Parker, P. J., Kour, G., Marsis, R., Mitchell, F., Pears, C., Schaap, D., Stabel, S., and Webster, C. (1989) Mol. Cell. Endocrinol. 65, 1-11
7. Isakov, N., McMahon, P., and Altmann, A. (1989) J. Biol. Chem. 264, 2091-2097
8. Aihara, H., Asaoke, Y., Yoshida, K., and Nishizuka, Y. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 11092-11096
9. Kiley, S. C., Parker, P. J., Fabbro, D., and Jaken, S. (1991) J. Biol. Chem. 266, 23761-23768
10. Brick-Ghannam, C., Huang, F. L., Temine, N., and Charron, Kiley, S. C., Parker, P. J., Fabbro, D., and Jaken, S. (1991) J. Biol. Chem. 266, 23761-23768
11. Herrmann, B. (1988) Nature 328, 445-449
12. Jaskolski, D., Daniel, K. J., Mercer, E., Calabretta, B., and Baserga, R. (1988) Science 244, 1544-1546
13. Godson, C., Ramirez, M. T., Baxter, G., Strulovici, B., and Insel, P. A. (1991) FASEB J. 5, A53
14. Ch'ng, J. L. C., Tolligan, R. C., Schimmel, P., and Holmes, E. W. (1989) Proc. Natl Acad. Sci. U. S. A. 86, 10006-10101
15. Insel, P. A., Weiss, R. A., Slivka, S. B., Howard, M. J., Waite, J. J., and Godson, C. (1991) Biochem. Soc. Trans. 19, 329-333
16. Clark, J. D., Milona, N., and Knopf, J. L. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 7076-7102
17. Kramer, R. M., Roberts, E. F., Manetta, J., and Putnam, J. E. (1991) J. Biol. Chem. 266, 5266-5272
18. Clark, J. D., Lin, L.-L., Kritz, R. W., Ramesha, C., Sultzman, L. A., Lin, A. Y., Milona, N., and Knopf, J. L. (1991) Cell 66, 1043-1051
19. Sharp, J. D., White, D. L., Chou, K. G., Goodson, T., Gao, B. C., McCutle, D., Burgett, S., Hoskins, J. A., Skatrud, P. L., Stack, J. R., Becker, G. W., Kung, L. H., Roberts, E. F., and Kramer, R. M. (1991) J. Biol. Chem. 266, 14850-14853
20. Clarke, M. A., Ozgur, L. E., Conway, T. M., Dispoto, J., Crooke, S. T., and Bormani, J. N. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 5418-5422
21. Togai, T. B., Rothot, B., Shaw, M., Prad, M., Vargaftig, B. B., and Russo-Marie, F. (1989) Nature 341, 177-180
22. Carter, T. D., Hallam, T. J., and Pearson, D. J. (1989) Biochem. J. 262, 431-437
23. Burch, R. W., Looney, A., and Axetold, J. A. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 7201-7205
24. Boventre, J. V., Gronich, B., Sessa, C., and Axelrod, J. A. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 6494-6498
25. Weiss, R. A., and Insel, P. A. (1991) J. Biol. Chem. 266, 2126-2133