Inhibitory Properties of the Regulatory Domains of Human Protein Kinase Ca and Mouse Protein Kinase Cε*

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Two fusion proteins in which the regulatory domains of human protein kinase Ca (Rα; amino acids 1–270) or mouse protein kinase Ce (Rε; amino acids 1–385) were linked in frame with glutathione S-transferase (GST) were examined for their abilities to inhibit the catalytic activities of protein kinase Ca (PKCa) and other protein kinases in vitro. Both GST-Rα and GST-Rε but not GST itself potently inhibited the activities of lipid-activated rat brain PKCa. In contrast, the fusion proteins had little or no inhibitory effect on the activities of the Ser/Thr protein kinases cAMP-dependent protein kinase, cGMP-dependent protein kinase, casein kinase II, myosin light chain kinase, and mitogen activated protein kinase or on the src Tyr kinase. GST-Rα and GST-Rε, on a molar basis, were 100–200-fold more potent inhibitors of PKCo activity than was the pseudosubstrate peptide PKCa19–36. In addition, a GST-Rα fusion protein in which the first 32 amino acids of Rα were deleted (including the pseudosubstrate sequence from amino acids 19–31) was an effective competitive inhibitor of PKCa activity. The three GST-R fusion proteins also inhibited protease-activated PKCa and proteolytically activated PKCo (PKM), two lipid-independent forms of PKCo; however, the IC50 values for inhibition were 1 order of magnitude greater than the IC50 values obtained in the presence of lipid. These results suggest that part of the inhibitory effect of the GST-R fusion proteins on lipid-activated PKCo may have resulted from sequestration of lipid activators. Nonetheless, as evidenced by their abilities to inhibit the lipid-independent forms of the enzyme, the GST-R fusion proteins also inhibited PKCa catalytic activity through direct interactions. These data indicate that the R domains of PKCa and PKCe are specific inhibitors of protein kinase Ca activity and suggest that regions of the R domain outside the pseudosubstrate sequence contribute to autoinhibition of the enzyme.

The protein kinase C (PKC) family is composed of Ca2+- and phospholipid-dependent isozymes that play important roles in signal transduction in both lower and higher eukaryotic cells. In mammalian cells the PKC family has been implicated in the regulation of a host of cellular processes including growth, secretion, ion channel conductance, gene expression, and receptor regulation (1–3). Each PKC isoform contains a catalytic (C) domain that catalyzes the phosphorylation of specific Ser and Thr residues and an regulatory (R) domain that inhibits the activity of the C domain via intramolecular interactions (for review, see Ref. 1). Some forms of PKC can be activated by receptor-mediated production of diacylglycerol, which binds to cysteine-rich sites within the R domain (4–6), and by Ca2+, which acts through high-affinity Ca2+-binding sites in the R domain (7). The binding of diacylglycerol, phosphatidylycerine, and Ca2+ to the R domain induces a conformational change that relieves the inhibitory effect of a pseudosubstrate-like sequence on catalytic activity. Evidence supporting this hypothesis comes from experiments in which a small peptide corresponding to the pseudosubstrate-like sequence within the R domain of PKCa significantly inhibited PKC catalytic activity (8), antibodies raised against this peptide constitutively activated the enzyme (9), and mutagenesis of sequences within the pseudosubstrate site of the PKC R domain resulted in partial activation of the enzyme (10). Furthermore, allosteric activation of the enzyme has been shown to expose the pseudosubstrate region of PKC to proteolytic attack consistent with its removal from the active site of the enzyme (11).

Recently we examined the effects of the entire R domain of PKC on PKC activity in vitro and demonstrated that the R domain of human PKCa (amino acids 1–270), when expressed as a fusion protein with GST, behaved as a potent competitive inhibitor of PKC catalytic activity (12). We also showed that the PKCa R domain inhibited PKC-mediated phenotypes in intact yeast cells and suggested that PKCa R might provide a useful reagent to achieve specific, dominant inhibition of PKC (12). In this study, we have examined the specificity with which the R domains from human PKCs and mouse PKCe inhibit PKC activity. We demonstrate that the R domains of PKCa and PKCe potently inhibit PKCe activity but do not appreciably inhibit the activities of other Ser/Thr or Tyr kinases tested. We also find that the deletion of the pseudosubstrate sequence from PKC Rα does not markedly diminish its ability to inhibit PKC holoenzyme or proteolytically activated PKCo (PKM) activity. On this basis, we suggest that R domain sequences

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‡ The abbreviations used are: PKC, protein kinase C; R domain, regulatory domain; C domain, catalytic domain; PKM, proteolytically activated PKCo; GST, glutathione S-transferase; PMA, phorbol 12-myristate 13-acetate.
outside the pseudosubstrate region of PKC may contribute significantly to enzyme autoinhibition.

MATERIALS AND METHODS

Reagents—Mitogen-activated protein kinase (from 
*Pisaster ochraceus*), human recombinant src kinase, and their peptide substrates (APRTPGGR and KVEKIGEGTGYGVYK, respectively) were purchased from Upstate Biotechnology Inc. (Lake Placid, NY). The PKCα substrate [Ser]-PKCα13–31 (RFARKGSRKTVN) and the PKCα pseudosubstrate peptide PKCα13–38 (RFARKGLIQNKNVYKVN) were obtained from Canadian Life Technologies Inc. (Burlington, Ontario, Canada). LRRASLG (Kemptide), PMA, purified rat brain PKCα, and isopropylthio-β-D-galactoside were obtained from Sigma, and PKM was obtained from Calbiochem-Novabiochem (San Diego, CA). Recombinant human casein kinase II and casein kinase II substrate peptide (RRREEETEEE) were from New England Biolabs (Mississauga, Ontario, Canada). The catalytic subunit of cAMP-dependent protein kinase from bovine aorta were purchased from Promega Corp. (Madison, WI). The myosin light chain kinase-specific substrate K-MLC11-23 (KKRRPPQRSVNSVF) was from Peninsula Laboratories (Belmont, CA). Chicken gizzard myosin light chain kinase and bovine brain calmodulin were generous gifts from Michael P. Walsh (University of Guelph, Ontario, Canada). Radiolabeled [*-32P]ATP (111 TBq/mmol) was from Amersham (Oxford, U.K.).

Preparation of GST-Rα, GST-Rα31–32, and GST-Rε—A fusion protein in which amino acids 1–270 of human PKCα was fused in frame to GST (GST-Rα) was prepared as described by Parissenti et al. (12) except that one tablet containing a wide spectrum of protease inhibitors (Complete, Roche Applied Science) was added per 50 ml of bacterial cell lysate and elution off glutathione-Sepharose columns was achieved by several overnight incubations with 50 mM glutathione at 4 °C. A GST fusion protein in which the first 32 amino acids of PKC R domain was deleted (GST-Rα31–32) was prepared by cloning a DreiII-EcoRI fragment of the human PKCα R domain in pbLuxExpress SK (12) into the GST fusion protein expression vector pGEX-5X (Amersham Pharmacia Biotech) using standard cloning techniques. A GST fusion protein containing the R domain of PKCα (amino acids 1–385) was deleted by cloning an Ncol-PvuII fragment from mouse PKCα cDNA in pMT2e (a gift from Dr. John Knowp, Genetics Institute, Cambridge, MA) into the Smal and EcoRI sites of the GST fusion vector pGEX-2T (Amersham Pharmacia Biotech) using standard cloning techniques. Construction of each of the expression vectors was confirmed by restriction endonuclease digestion and DNA sequencing. These fusion proteins also were purified from *Escherichia coli* DH5α as described above. The GST-R fusion proteins were dialyzed in 1 mM EDTA, 1 mM dithiothreitol, and 10 mM Hepes, pH 7.5, and stored frozen at −70 °C.

Immunoblot Analysis—Samples of GST-Rα, GST-Rα31–32, and GST-Rε in SDS sample buffer were electrophoresed on 10% polyacrylamide gels in the presence of SDS as described by Laemmli (13), and electroblotted onto nitrocellulose membranes. The membranes were then immunoblotted using a goat anti-rat brain PKC antibody (14) or a mouse monoclonal antibody specific for PKCε (Transduction Laboratories, Lexington, KY); antigen-antibody reactivity was detected using horseradish peroxidase-labeled secondary antibodies using the ECL detection system (Amersham Pharmacia Biotech).

Measurement of PKC, PKM, and Other Protein Kinase Activities—PKCα catalytic activity was measured by monitoring the transfer of [*-32P]ATP from [*-32P]ATP to the peptide substrate [Ser]-PKCα13–31 (8) in 30 min reactions at 30 °C. Unless otherwise indicated, reactions (100 μl) contained 2 μM [Ser]-PKCα13–31, 164 μM [*-32P]ATP (0.5 μCi), 18 mM MgCl2, 2 mM CaCl2, 46.4 μg/ml phosphatidylserine, 2.5 μM PMA, 20 mM Tris-HCl, pH 7.5, and 5 μl of purified rat brain PKCα. The phosphatidylserine and PMA were added together as a sonicated emulsion to form small unilamellar vesicles. Assays of PKM activity were conducted under similar conditions except that the concentration of [Ser]-PKCα13–31 was 1 μM, and 1 mg/ml bovine serum albumin was added to stabilize the enzyme, and reactions were 12 min. Reactions were terminated by boiling 90 μl of sample onto P81 phosphocellulose paper filters. Filters were washed with 1% phosphoric acid and counted by liquid scintillation spectrometry.

Other Ser/Thr kinase activities and src Tyr kinase activity were assayed by measuring the transfer of [*-32P]ATP from [*-32P]ATP to acceptor peptide substrates under standard conditions for each enzyme. In each case, peptide substrate concentrations were equal to experimentally determined Km values. cAMP-dependent protein kinase activity was measured by incubating the catalytic subunit of the enzyme (25 ng) with 20 μM Kemptide (15) for 5 min at 30 °C in a reaction (50 μl) containing 5 mM MgCl2, 1 mM EGTA, 100 μM [*-32P]ATP (1 μCi), and 20 mM Tris- HCl, pH 7.5. cGMP-dependent protein kinase assays were conducted under identical conditions to that described for cAMP-dependent protein kinase except that 4 μM Kemptide and 2 μM cGMP were present in the reaction. Mitogen-activated protein kinase kinase activity was assayed by incubating the enzyme (13.5 ng) with 1.5 mM mitogen-activated protein kinase substrate peptide for 5 min at room temperature in a reaction (25 μl) containing 12.5 mM β-glycerolphosphate, 7.5 mM MgCl2, 0.5 mM EGTA, 2 mM dithiothreitol, 50 μM NaF, 0.5 mM sodium orthovanadate, 50 μM [*-32P]ATP (1 μCi), and 12.5 mM 3-(N-morpholino)propanesulfonic acid, pH 7.2 (16). Myosin light chain kinase activity was measured by incubating the enzyme (25 ng) with 20 μM K-MLC11–23 Substrate peptide (17) for 4 min at 30 °C in a reaction (100 μl) containing 4 mM MgCl2, 60 mM KCl, 150 μM CaCl2, 1 mg of calmodulin, 200 μM [*-32P]ATP (0.8 μCi), and 25 mM Tris-HCl, pH 7.5. Casein kinase II activity was measured by incubating the enzyme (0.5 milliunit) with 500 μM substrate peptide (18) for 5 min at 37 °C in a reaction (50 μl) containing 130 mM KCl, 10 mM MgCl2, 4.8 mM dithiothreitol, 50 μM [*-32P]ATP (1.2 μCi), and 20 μM 2-(N-morpholino)ethanesulfonic acid, pH 6.9. src kinase assays were conducted by incubating 6 units of purified enzyme with 100 μM substrate peptide (cde2–20) for 10 min at room temperature in a reaction (50 μl) containing 2.5 mM MnCl2, 0.5 mM EGTA, 0.625 mM sodium orthovanadate, 31 mM sodium acetate, 31 mM MgCl2, 112 μM [*-32P]ATP (1 μCi), and 25 mM Tris- HCl, pH 7.2. Reactions were terminated and processed as described above for PKC.

RESULTS

Isolation and Characterization of GST-Rα, GSTRα31–32, and GST-Rε—Purified preparations of GST-Rα, GST-Rα31–32, and GST-Rε yielded major protein bands on SDS-polyacrylamide gels that migrated with apparent molecular weights in reasonable agreement with their predicted masses (56 kDa for GST-Rα, 52 kDa for GST-Rα31–32 and 68 kDa for GST-Rε) including 26 kDa for the GST component (Fig. 1). GST-Rα and GST-Rα31–32 also reacted with the antibody that recognizes the α, β,
and γ isoforms of rat PKC (14), whereas GST-Re reacted with the PKCe antibody (Fig. 1), thus confirming the identity of the fusion proteins. The GST-Re preparation contained a small amount of contaminating low molecular weight material that was visible on stained gels; presumably, this material represents a proteolytic product of GST-Re, as it is strongly immunoreactive with the monoclonal PKCe antibody and has a molecular weight equal to the R domain of PKCe without GST attached.

**Effects of GST-Ro and GST-Re on PKCa Activity and on the Activities of Various Other Ser/Thr and Tyr Kinases—**Previously we demonstrated that GST-Ro is a potent competitive inhibitor of yeast-expressed bovine PKCa and yeast-expressed rat PKCβ-1 (12). To assess whether the inhibition of protein kinase activity by GST-Ro was PKCa-specific, we examined the ability of GST-Ro to inhibit the activities of several Ser/Thr and Tyr kinases. As shown in Fig. 2A, GST-Ro potently inhibited the activity of purified rat brain PKCa (IC50 = 40 nM) but did not inhibit the activities of cAMP-dependent protein kinase, cGMP-dependent protein kinase, or myosin light chain kinase and only marginally inhibited the activities of casein kinase II, mitogen activated protein kinase, and src kinase. In fact, GST-Ro stimulated the activities of cGMP-dependent protein kinase and myosin light chain kinase, although the mechanism of this activation by GST-Ro is unknown. To determine whether the effects of GST-Ro were isozyme-specific, we examined the effects of GST-Re on PKCa activity. GST-Re (Fig. 2B) also inhibited PKCa activity (IC50 = 60 nM) while having little or no effect on the other Ser/Thr or Tyr kinases described above. As determined from double-reciprocal plots of enzyme *versus* substrate concentration, GST-Ro and GST-Re each competitively inhibited PKCa activity with *Km* values of 0.5 ± 0.03 μM (Fig. 3A) and 0.8 ± 0.4 μM (Fig. 3B), respectively. GST alone at concentrations up to 10 μM did not inhibit PKCa activity (Fig. 4), nor did it inhibit the activities of any of the other protein kinases tested above (data not shown). These data indicate that the R domains of PKCa and PKCe are selective, competitive inhibitors of PKC activity but do not exhibit PKC isoform selectivity when assayed in *vitro* in the presence of activating lipids.

**Role of the Pseudosubstrate Sequence in the Inhibition of PKCa by GST-Ro—**Although the R domains of PKCa and PKCe have different pseudosubstrate sequences, their lack of selectivity for PKC isozymes (Fig. 2) could be reconciled, as four amino acid residues within the PKCa and PKCe pseudosubstrate sequences are conserved (19), including Arg-22, which is essential for the inhibitory activity of the PKCa pseudosubstrate peptide (20). On a molar basis, however, the GST-Ro and GST-Re fusion proteins inhibited PKC activity 100–200 times more potently than did the PKC pseudosubstrate peptide PKC19–36 (Fig. 4), which has a *Ks* for PKCa of 26 μM (data not shown). These observations raised the possibility that regions within the PKC R domain but outside the pseudosubstrate site play important roles in the inhibition of PKC activity. To test this hypothesis further, a fusion protein was prepared in which amino acids 1–32 of the PKCa R domain were deleted and the remaining sequence (amino acids 33–270) was linked in frame with GST. This protein, GST-Rota1–32, which lacks the pseudosubstrate sequence, retained the ability to inhibit the activity of purified rat brain PKCs over a concentration range similar to that seen for GST-Re and GST-Re (Fig. 4); inhibition of PKCa activity by GST-Ro1–32 was competitive (Fig. 3C; *Ks* = 0.25 ± 0.12 μM). These observations strongly suggest that regions within the PKC R domain but outside the pseudosubstrate sequence play a significant role in the inhibition of PKC catalytic activity.

**Effects of GST-R Fusion Proteins on PKM and Protamine-activated PKCa Activities—**We next examined the effects of GST-Ro, GST-Rota1–32 and GST-Re on protamine-activated PKCa and on PKM to distinguish direct effects of the fusion proteins on PKCa from indirect effects due to sequestration of lipids. Protamine serves both as a substrate for PKC and as an allosteric activator of the enzyme, thus obviating the need for phosphatidylserine, phorbol ester, or Ca2⁺ as activators. In the assay (21), PKM is a purified proteolytic product of PKCa that contains only the catalytic half of the protein and neither requires nor is responsive to phosphatidylserine, phospholipid, or Ca2⁺. As shown in Fig. 5A, GST-Re inhibited protamine-activated PKCa in a concentration-dependent manner with an *IC50* value of 650 ± 80 nM (n = 3); GST-Rota1–32 and GST-Re also inhibited protamine-activated PKC but with somewhat higher *IC50* values of 1050 ± 60 nM (n = 3) and 1300 ± 100 nM (n = 3), respectively. GST alone did not inhibit protamine-activated PKCa. The inhibitory effects of the GST-R fusion proteins could be overcome with higher concentrations of protamine (data not shown), suggesting that the GST-R fusion proteins were com-

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**Fig. 2. Effects of GST-Ro and GST-Re on the catalytic activities of various protein kinases.** Increasing concentrations of purified GST-Ro (A) and GST-Re (B) were examined for their abilities to inhibit the catalytic activities of purified rat brain PKCa (●), cAMP-dependent protein kinase (▲), casein kinase II (▲), cGMP-dependent protein kinase (○), myosin light chain kinase (●), and src kinase (△) at substrate concentrations equal to the experimentally determined *Km* for each enzyme. Activities are presented as percentages of the total activity present in the absence of fusion protein and are representative of three independent experiments.
petitive with substrate; however, the double-reciprocal plots of velocity versus protamine concentration were parabolic in the absence or presence of PKCα R, consistent with positive cooperative effects of protamine on PKC activity observed previously (12, 21). The GST-R fusion proteins were considerably more potent inhibitors of protamine-activated PKCα than was the pseudosubstrate peptide PKC19–36, as the pseudosubstrate peptide did not inhibit protamine-activated PKCα (Fig. 5A) even at concentrations as high as 50 μM. This finding is consistent with the data presented in Fig. 4 which shows that the GST-R fusion proteins inhibit PKCα activity with potencies that are 100–200-fold greater than PKC19–36. GST-RaD1–32 and GST-Re also inhibited PKM activity with IC50 values of approximately 500 nM (Fig. 5B). As expected, phosphatidylserine, PMA, and Ca2+ had little effect on PKM activity (data not shown). These observations thus indicate that the inhibition of PKCα activity by GST-R fusion proteins may result at least in part from direct effects of the fusion proteins on the catalytic domain of the enzyme.

**DISCUSSION**

As shown in this in vitro study, GST-Rα and GST-Re inhibited lipid-activated PKCα activity effectively and with equal potency but did not inhibit the activities of several other Ser/Thr protein kinases or the src Tyr kinase (Figs. 2 and 4). Thus the GST-R fusion proteins displayed a high degree of specificity for PKC but lacked appreciable PKC isozyme selectivity under these in vitro conditions. Although a number of observations indicate that the R domains of PKC contribute to the selective actions of different PKC isozymes, isozyme selectivity seems to depend upon the responsiveness of the PKC isoforms to different activating ligands and the targeting of the PKCs to different subcellular compartments (23–25), factors that were not assessed in the in vitro assays of PKC activity described here.

The inhibition of lipid-activated PKCα by the GST-R fusion proteins may have reflected competition between GST-R and substrate for the active site of PKC (8) or may have been secondary to the sequestration of lipid cofactors. The sequestration of lipids might impact on PKC activity by reducing the effective concentrations required for activation of PKC, by reducing the electrostatic potential of the vesicles thereby preventing PKC binding to lipid surfaces (1), or by inhibiting delivery of the PKC substrate peptide to the enzyme (26). Indeed, lipids appeared to contribute to the inhibitory potency of the GST-R fusion proteins, because the IC50 values of the
GST-R fusion proteins were 1 order of magnitude lower in lipid-dependent assays of PKCa activity compared with the lipid-independent assays of PKC activity (Fig. 4 versus Fig. 5). Although the concentrations of phosphatidylserine (approximately 50 μM) in the PKC assays were approximately 1000-fold higher than the concentrations of fusion proteins required for 50% inhibition of lipid-activated PKCa (approximately 50 nM), the regulatory domain constructs may have sequestered up to 12 times the amount of phosphatidylserine as estimated from direct PKC-lipid binding assays (27) and may have sterically masked access to 100 times the amount of lipid as estimated from light scattering and fluorescent energy transfer measurements (28). Reductions in surface electrostatic surface potential may have had additional effects on the interaction of PKC with lipid surfaces. Therefore, it is possible that the increased inhibitory potency of the GST-R fusion proteins on lipid-activated PKC activity resulted from a nonselective sequestration of lipids required for enzyme activation. Nonetheless, the GST fusion proteins inhibited lipid-independent activated forms of PKCa (Fig. 5), indicating that the GST fusion proteins can directly inhibit the catalytic domain of the protein. Furthermore, in the absence of lipid, GST-Ra inhibited PKCa activity with a 2-fold greater potency than GST-Re or GST-Rb (Fig. 5), suggesting a modest degree of isotype selectivity and a modest role for the pseudosubstrate region in the inhibition of PKC activity by GST-R under these in vitro conditions.

Although there is considerable evidence that the pseudosubstrate region of PKC plays a role in the autoinhibition of the enzyme, our observations raise the possibility that other regions in the PKC R domain have significant PKC inhibitory activity. We find that the R domain of PKCa is a substantially more potent PKC inhibitor than is the pseudosubstrate peptide PKC19–36 either in the presence of absence of lipid activators and that the R domain of PKCa retains significant PKC inhibitory activity after removal of the pseudosubstrate site (Figs. 4 and 5). Additional support for this hypothesis comes from observations of Riedel et al. (29). This group found that deletion of the N-terminal 153 amino acids of bovine PKCα, including the pseudosubstrate and phorbol ester-binding domains, led to increased PKC constitutive activity and a loss of phorbol ester-activated enzyme activity, consistent with an autoinhibitory function for the pseudosubstrate site. They also observed, however, that the truncated enzyme could be further activated 2.5–3-fold by the addition of Ca2+. Although they did not comment on the significance of this finding, their results are consistent with our suggestion that domains outside the pseudosubstrate site contribute to the negative regulation of PKC by its R domain. One candidate region that might function to inhibit PKC activity is the pseudoRACK site, which is found in the R domains of different PKC isoforms (30). The pseudoRACK site has sequence similarity to receptors for activated PKC (RACKS) that participate in the targeting of different PKC isoforms to selective substrates and is conserved in different PKC isoforms. These pseudoRACK sites are proposed to interact with RACK binding sites which overlap parts of the catalytic domain of PKC and may contribute to enzyme autoinhibition.

Recently expression vectors encoding the regulatory domains of different PKC isoforms have been used in transfection studies to assess the roles of PKC in signal transduction. Expression vectors encoding Rα, Rβ1, and Rδ were shown to affect the growth of rat embryo fibroblasts (31, 32), whereas an expression vector encoding Rε inhibited Golgi functions in mouse fibroblasts (33). In these studies, the mechanisms by which the R domain proteins exerted their effects were not explored, although they were presumed to act as dominant inhibitors of PKC by interfering with PKC substrate utilization or with interactions of PKC with its binding proteins. As demonstrated here, PKC R domain proteins inhibit substrate phosphorylation with marked specificity through direct effects on the enzyme and possibly through effects associated with sequestration of lipid activators. Although Rα and Rε exhibited only modest PKC isoform selectivity in vitro (Fig. 5), it remains possible that Rα and Rε might behave as isoform-specific inhibitors of PKC in vivo, for example by preventing the translocation of the corresponding PKC isozyme to specific subcellular compartments. Interestingly, Rα and Rε each contain regions corresponding to RACK binding sites that have been shown previously to interfere with the subcellular localization of specific PKC isoforms (23, 34).

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Fig. 5. Inhibitory effects of GST-R fusion proteins on protamine-activated PKCa and PKM activities. Increasing concentrations of GST-Rα (●), GST-Re (■), GST-Rb (□), or GST-C19–36 (▲) were evaluated for their abilities to inhibit protamine-activated PKCa from rat brain (A). Reactions (100 μl) contained 100 μM [γ-32P]ATP (0.5 μCi), 10 mm MgCl2, 250 μM EGTA, 30 mm NaCl, 4 μM GST, 2.1 μM protamine, and 5 milliliters of purified PKCa. Samples were assayed for PKC activity as described under “Materials and Methods.” Results are expressed as a percentage of the maximal activity obtained in the absence of inhibitor ± SE (n = 3). Increasing concentrations of GST-Rb (▲), GST-ε (■), and GST-Re (□) also were tested for their effects on the activity of the proteolytically activated form of PKCa, PKM (B). PKM assays were conducted as described under “Materials and Methods.” Results are expressed as nanomoles of 32P transferred from [γ-32P]ATP to (Ser-25)PKC19–31/min/mg of protein.

A

B

Concentration of agent, μM

Protein kinase activity (%)

Concentration of agent, μM

Protein kinase activity
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