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# Widespread occurrence of "87 kDa," a major specific substrate for protein kinase C

(phosphoprotein/phosphorylation/nervous system)

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ABSTRACT **An 87-kDa phosphoprotein, identified previously as a major, specific substrate for $Ca^{2+}$/phospholipid/diacylglycerol-dependent protein kinase (protein kinase C) in broken cell preparations from rat brain, has been characterized with respect to its species, tissue, and subcellular distribution. A similar protein was present in monkey, human, mouse, and bovine brain and in *Torpedo californica* electric organ. The protein was also identified in a variety of non-neuronal rat and bovine tissues. The rat protein had an apparent molecular mass 4–7 kDa lower, and was slightly more acidic, than the protein in bovine tissues. The 87-kDa proteins from various bovine tissues were identical by the following criteria: each was phosphorylated by exogenous protein kinase C, was of comparable molecular mass, generated multiple spots within the pH range of 4.4–4.9 upon isoelectric focusing, yielded identical patterns upon digestion with *Staphylococcus aureus* V8 protease, and was recognized by a specific 87-kDa antiserum. The relative concentrations of the 87-kDa protein in bovine tissues were highest in brain, spleen, and lung, moderate in testis, pancreas, adrenal, kidney, and liver, and lowest in heart and skeletal muscle. In the brain, the 87-kDa protein was concentrated in the synaptosomal membrane and in the cytosol. The membrane-bound protein was extractable with nonionic detergents but not with NaCl. This species, tissue, and subcellular distribution of the 87-kDa protein is similar to that of protein kinase C.**

A wide variety of neurotransmitters, hormones, and other biologically active substances, which are known to stimulate inositol phospholipid breakdown and to generate diacylglycerol, are believed to mediate many of their functions through activation of $Ca^{2+}$/phospholipid/diacylglycerol-dependent protein kinase (protein kinase C). Tumor-promoting phorbol esters are also able to bind to and activate protein kinase C. This protein phosphorylation system appears to be involved in widespread functions, including events in cellular proliferation and, in synergism with $Ca^{2+}$ mobilization, hormone and neurotransmitter release (for review, see ref. 1).

There is little information about the biochemical events mediating the proposed connection between protein kinase C activation and cellular responses. It is important, therefore, to identify and characterize physiological substrates for protein kinase C. A protein of 87 kDa was shown to be a substrate for protein kinase C in broken cell preparations from rat brain (2) and to be phosphorylated in response to depolarization-induced $Ca^{2+}$ influx (2) and tumor-promoting phorbol esters (3) in intact nerve terminals from rat brain. This 87-kDa substrate was also shown to be present in all regions of the rat brain (4, 5). In the present study, we characterized the species, tissue, and subcellular distribution of this 87-kDa protein.

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## MATERIALS AND METHODS

**Materials.** Frozen cerebral cortex from macaque monkey brain was a gift from Tamas Bartfai (Stockholm, Sweden). Fresh rat tissues were dissected from stunned and decapitated male Sprague–Dawley rats. Fresh bovine tissues were obtained from a local slaughterhouse and were transported to the laboratory either on ice, in liquid nitrogen, or as a preparation homogenized in boiling 1% $NaDodSO_4$. Protein kinase C was purified to homogeneity from bovine cerebral cortex by modification of the procedure of Kikkawa *et al.* (6). The soluble 87-kDa protein was purified as described (7) except that the final step was chromatography on a fast protein liquid chromatography Mono Q column (Pharmacia). Antiserum against the 87-kDa protein was prepared in a New Zealand female rabbit by intradermal injection into multiple sites of 75 μg of the purified protein emulsified in complete Freund's adjuvant. The rabbit was given a booster injection with 50 μg of 87-kDa protein at 7 weeks after the first injection and was bled at weekly intervals thereafter. The bleeding at 9 weeks was used for the present study. The antiserum was specific for the 87-kDa protein. The heat-stable protein inhibitor of cAMP-dependent protein kinase ("Walsh inhibitor") was purified by a modification of the published procedure (8) and was a gift from Angus Nairn.

ATP, bovine serum albumin, $CaCl_2$, diolein, dithioerythritol, EDTA, EGTA, H1 histone (III-S), 2-mercaptoethanol, molecular mass protein standards for gel electrophoresis, Nonidet P-40 (NP-40), L-α-phosphatidyl-L-serine, and Tris were purchased from Sigma. Other materials were purchased from the following commercial sources: [$\gamma$-$^{32}$P]ATP and $^{125}$I-labeled protein A (New England Nuclear); calmidazolium (Boehringer Mannheim); chymostatin and leupeptin (Chemicon, El Segundo, CA); Hepes and phenylmethylsulfonyl fluoride (Calbiochem–Behring); magnesium acetate (Baker); nitrocellulose, pore size, 0.2 μm (Schleicher & Schuell); $NaDodSO_4$ (Bio-Rad); *Staphylococcus aureus* V8 protease (Miles); and Trasylol (FBA Pharmaceuticals, New York). All other chemicals used were reagent grade.

**Preparation of Tissue Samples for Phosphorylation.** Monkey cerebral cortex (from storage at −70°C), fresh rat cerebral cortex, and bovine tissue samples (from storage in a liquid nitrogen freezer) were homogenized at 0–4°C for 60 sec with a Polytron in a 10× volume of 20 mM Hepes at pH 7.4, 100 mM NaCl, 2 mM EDTA, 0.5 mM EGTA, 16 μg of chymostatin per ml, 10 μg of leupeptin per ml, 100 μM phenylmethylsulfonyl fluoride, and Trasylol (50 Kallikrein inactivator units/ml) and filtered through cheesecloth. Protein concentration was determined by the method of Bradford (9).

**Phosphorylation of Endogenous Substrates for Protein Kinase C.** The standard reaction mixture (final volume, 100 μl) contained 20 mM Tris·HCl at pH 7.4, 10 mM magnesium acetate, 1.0 mM EGTA, 2 mM dithioerythritol, 0.72 μg of Walsh inhibitor, 5 μM calmidazolium, 10 μg of leupeptin, and

Abbreviation: NP-40, Nonidet P-40.

100 μg of tissue protein, in the absence or presence of 1.5 mM $CaCl_2$, 5 μg of phosphatidylserine, 0.2 μg of diolein, and 4 ng of protein kinase C (specific activity, 2 μmol of $^{32}P$ incorporated per min/mg of protein, with H1 histone as substrate, measured under standard assay conditions). The reaction was initiated by the addition of [γ-$^{32}P$]ATP (final concentration, 2 μM; specific activity, 22 × $10^6$ cpm/nmol) and was carried out for 60 sec at 30°C. The reaction was terminated by the addition of 20 μl of $NaDodSO_4$ stop solution [20% (vol/vol) glycerol/10% (wt/vol) $NaDodSO_4$/10% (wt/vol) 2-mercaptoethanol/0.25 M Tris·HCl, pH 6.7, containing a trace of bromophenol blue] and heating in a boiling water bath for 5 min.

Proteins (50 μg) in the samples were separated by one-dimensional electrophoresis on $NaDodSO_4$/6–12% mixed exponential-linear gradient polyacrylamide gels by the method of Kelly and Luttges (10). The gels were stained, destained, dried, and subjected to autoradiography as described (11). Radioactivity in phosphorylated proteins was quantitated with an AMBIS beta scanning system (Automated Microbiology Systems, San Diego, CA).

For two-dimensional gel electrophoresis, proteins (50 μg) in the samples were precipitated with 90% ice-cold acetone, solubilized, and separated by the method of O'Farrell (12), using isoelectric focusing on a pH 3.5–5.8 gradient in the first dimension.

**Immunoprecipitation of 87-kDa Protein.** Proteins in samples were phosphorylated as described above except that the reaction was terminated by $NaDodSO_4$ (final concentration, 1%). Immunoprecipitation was performed with minor modifications of the described procedure (13) except that 40 μl of 87-kDa protein antiserum was used. Proteins in the final supernatant were subjected to $NaDodSO_4$/polyacrylamide gel electrophoresis. The gels were stained, destained, dried, and subjected to autoradiography as described (11). Radioactivity in phosphorylated proteins was quantitated with the AMBIS beta scanning system. Gel pieces containing phosphorylated protein bands were subjected to limited proteolysis with *S. aureus* V8 protease as described (14).

**Immunoblotting of 87-kDa Protein.** Fresh bovine tissues were homogenized at the slaughterhouse with an Ultra-Turrax in a 10× volume of boiling 1% $NaDodSO_4$ and heated for another 5 min in a boiling water bath. Protein concentration was determined by the method of Peterson (15). Proteins (50 μg) in the samples were separated by one-dimensional electrophoresis on $NaDodSO_4$/6–12% mixed exponential-linear gradient polyacrylamide gels. The proteins were then transferred to nitrocellulose sheets at 350 mA for 12 hr using the buffer system of Towbin *et al.* (16). Immunoblotting was performed with $^{125}I$-labeled protein A as described (17) except that a 1:200 dilution of 87-kDa protein antiserum was used. Radioactivity in labeled proteins was quantitated with the AMBIS beta scanning system.

**Preparation of Subcellular Fractions.** Subcellular fractions were prepared from fresh bovine cerebral cortex as described (18). The crude synaptosomal fraction ($P_2$) was subdivided into particulate ($P_2$-P) and cytosol ($P_2$-S) fractions by hypotonic lysis in buffer [10 mM Hepes at pH 7.4, 2 mM EDTA, 1 mM dithioerythritol, and Trasylol (50 Kallikrein inactivator units/ml)] followed by centrifugation at 35,000 × *g* for 30 min. Extraction of proteins from the particulate fraction into the same buffer containing 0.5 M NaCl or 1% NP-40 was performed as described (19). Each particulate fraction was resuspended in a volume of buffer equal to that of the final supernatant ($S_3$). Proteins in aliquots (10 μl) of the fractions were separated by one-dimensional $NaDodSO_4$/6–12% mixed exponential-linear gradient polyacrylamide gel electrophoresis and subjected to immunoblotting as described above.

## RESULTS

**Species Distribution of the 87-kDa Protein.** The 87-kDa protein was observed as a major endogenous substrate for protein kinase C in homogenates from monkey, rat, and bovine brain (Fig. 1*A*) The molecular mass of the 87-kDa protein varied depending on the gel system used. In this $NaDodSO_4$/6–12% mixed exponential-linear gradient polyacrylamide gel, the protein from monkey and bovine brain had a molecular mass of 90 kDa, whereas that from rat brain had a molecular mass of 83 kDa (Fig. 1*A*). This difference was consistent, since in all experiments the phosphorylated 87-kDa proteins from rat or mouse (not shown) brain always had molecular masses 4–7 kDa lower than those from monkey, bovine, or human (not shown) brain. The 87-kDa antiserum precipitated a single phosphorylated protein from preparations from monkey, rat, and bovine brain (Fig. 1*B*) and from human brain (not shown). This immunoprecipitation confirmed that the protein from each species was similar immunologically and that the rat protein had a molecular mass 4–7 kDa lower than the proteins from the other species. When subjected to isoelectric focusing, the phosphorylated proteins from each species were found to be acidic with multiple (at least three) phosphorylated spots, with pI values ranging from 4.5 to 4.8 in the human, 4.4 to 4.8 in the monkey and bovine, and 4.3 to 4.6 in the rat (not shown). When subjected to limited digestion with *S. aureus* V8 protease or limit digestion with thermolysin, the 87-kDa protein from each species generated identical phosphopeptide maps (not shown). These V8 protease and thermolytic maps were also generated upon digestion of a similar protein from *Torpedo californica* electric organ (unpublished results

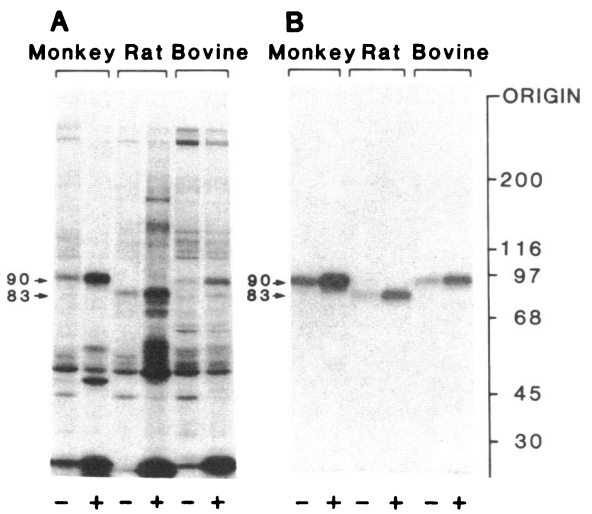


FIG. 1. (*A*) Autoradiogram showing phosphorylation of endogenous substrates for protein kinase C in homogenates of monkey, rat, and bovine brain. Phosphorylation was carried out under standard conditions in the absence (−) or presence (+) of calcium (500 μM free), phosphatidylserine (5 μg/100 μl)/diolein (0.2 μg/100 μl), and protein kinase C (4 ng). After termination of the reaction with $NaDodSO_4$ stop solution, samples (50 μg of protein) were subjected to one-dimensional electrophoresis on $NaDodSO_4$/6–12% mixed exponential-linear gradient polyacrylamide gels, followed by autoradiography. (*B*) Autoradiogram showing specificity of the immunoprecipitation of 87-kDa protein from monkey, rat, and bovine brain. Phosphorylation was carried out as in *A* except that the reaction was terminated with $NaDodSO_4$ (1% final concentration). Samples were subjected to immunoprecipitation with 40 μl of 87-kDa protein antiserum, followed by gel electrophoresis as in *A*. 90, Position of 87-kDa protein in monkey and bovine brain in this gel system; 83, position of 87-kDa in rat brain in this gel system. Positions of size markers are shown in kDa.

and R. Huganir, personal communication). The protein from *Torpedo californica* electric organ had the same molecular mass as the proteins from monkey, bovine, and human brain. Therefore, the only detectable differences between the 87-kDa proteins from different species were that the rodent protein had a molecular mass 4–7 kDa lower and was slightly more acidic than the proteins from the other species tested.

**Tissue Distribution of the 87-kDa Protein.** Endogenous proteins in homogenates of 10 bovine tissues were assayed with exogenous protein kinase C. The 87-kDa protein was a prominent substrate in the brain and lung and was visible as a substrate in the adrenal, spleen, and testis (Fig. 2*A*). When proteins in homogenates from the same tissues were subjected to immunoblotting, the 87-kDa protein was detected in all tissues examined (Fig. 2*B*), although the relative concentration varied among the tissues. It was highest in brain, spleen, and lung, moderate (about 30–50% of that in brain) in testis, pancreas, adrenal, kidney, and liver, and lowest (<10% of that in brain) in heart and skeletal muscle. Therefore, though the phosphorylation and immunoblotting results were generally in good agreement, they showed some differences in the apparent relative amounts of the 87-kDa protein in the various tissues—e.g., the spleen protein was less prominent by phosphorylation than by immunoblotting. Possible causes for these differences include masking of phosphorylated 87-kDa protein by other substrates of similar molecular mass, differences in ATPase, phosphoprotein phosphatase, or protein kinase inhibitor activities among the tissues, and differences in proteolytic activities among the tissues. Evidence for the latter possibility was obtained in some experiments when less fresh bovine tissues were used (prepared >5 hr after removal from the animal); in these cases a major proteolytic product of 47 kDa was detected by immunoprecipitation and immunoblotting.

The 87-kDa proteins from the various bovine tissues were identical by all criteria examined. When subjected to one-dimensional electrophoresis (on a $NaDodSO_4$/6–12% mixed exponential-linear gradient gel) followed by immunoblotting, each protein was seen as a broad band of 90 kDa (Fig. 2*B*). When subjected to isoelectric focusing, the phosphorylated protein from each tissue was found to be acidic with multiple spots (at least three) within the range of pI 4.4–4.9. Examples from cerebral cortex, testis, and adrenal are shown in Fig. 3. The pI of the major phosphorylated spot from each tissue varied from experiment to experiment; in the experiment shown, the major phosphorylated spot was more acidic in brain than in testis or adrenal (Fig. 3). This variability was probably the result of a posttranslational modification, most likely phosphorylation itself. The 87-kDa antiserum precipitated a single phosphorylated protein of similar molecular mass from each tissue. Examples from cerebral cortex, testis, and adrenal are shown in Fig. 4*A*; each protein had a molecular mass of 80 kDa in this $NaDodSO_4$/8% polyacrylamide gel. Limited proteolysis with *S. aureus* V8 protease of the immunoprecipitated phosphorylated protein from each tissue generated an identical phosphopeptide map, with major and minor phosphopeptides having molecular masses of 13 kDa and 9 kDa, respectively. Examples from cerebral cortex, testis, and adrenal are shown in Fig. 4*B*.

Analysis of rat tissues with similar techniques gave comparable results. Phosphorylation of endogenous substrates with exogenous protein kinase C showed that the 87-kDa protein was most prominent in brain, was prominent in lung, spleen, and vas deferens, and was detectable in testis, pancreas, and liver (data not shown). The molecular mass was 80 kDa when proteins were subjected to one-dimensional $NaDodSO_4$/7–12% linear gradient polyacrylamide gel electrophoresis. The 87-kDa antiserum precipitated a single 80-kDa phosphoprotein from each tissue. When subjected to isoelectric focusing, the phosphoprotein from each rat tissue generated multiple acidic spots (within the pH range of

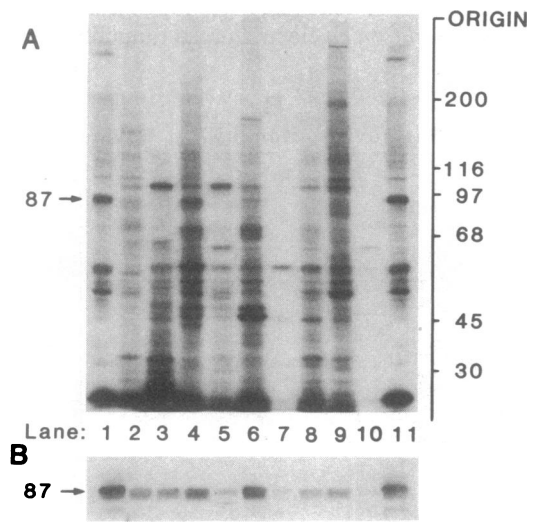


FIG. 2. (*A*) Autoradiogram showing phosphorylation of endogenous substrates for protein kinase C in homogenates of bovine tissues. Phosphorylation was carried out under standard conditions in the presence of calcium (500 μM free), phosphatidylserine (5 μg/100 μl)/diolein (0.2 μg/100 μl), and protein kinase C (4 ng/100 μl). After termination of the reaction with $NaDodSO_4$ stop solution, samples (50 μg of protein) were subjected to one-dimensional electrophoresis on $NaDodSO_4$/6–12% mixed exponential-linear gradient polyacrylamide gels, followed by autoradiography. Positions of size markers are shown in kDa. (*B*) Autoradiogram of immunoblot showing the distribution of 87-kDa protein in bovine tissues. Tissue samples were homogenized in a 10× volume of boiling 1% $NaDodSO_4$, and aliquots (50 μg of protein) were subjected to one-dimensional electrophoresis on $NaDodSO_4$/6–12% mixed exponential-linear gradient polyacrylamide gels. The proteins were transferred to nitrocellulose sheets and subjected to immunoblotting with a 1:200 dilution of 87-kDa protein antiserum followed by $^{125}I$-labeled protein A overlay. Lane numbers represent the following tissues: 1 and 11, cerebral cortex; 2, adrenal; 3, pancreas; 4, lung; 5, liver; 6, spleen; 7, heart; 8, kidney; 9, testis; 10, skeletal muscle. 87, Position of 87-kDa protein.

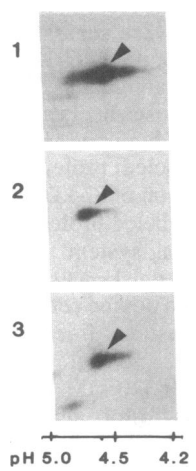


FIG. 3. Autoradiogram showing heterogeneity of 87-kDa protein upon isoelectric focusing. Phosphorylation was carried out as described in the legend to Fig. 2. After termination of the phosphorylation reaction, samples (50 μg of protein) were precipitated with acetone and subjected to two-dimensional electrophoresis using isoelectric focusing in the first dimension (pH range, 3.5–6) followed by electrophoresis on $NaDodSO_4$/6–12% mixed exponential-linear gradient polyacrylamide gels. Arrowheads, major radioactive species in each case. 1, Cerebral cortex; 2, testis; 3, adrenal.

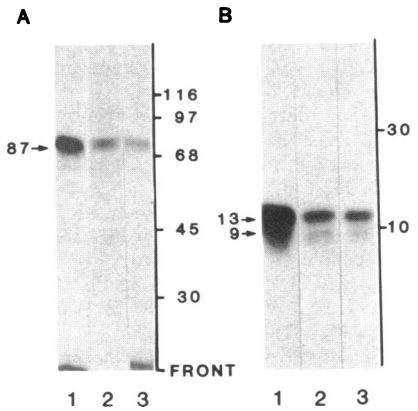


FIG. 4. (*A*) Autoradiogram showing specificity of the immunoprecipitation of 87-kDa protein from various bovine tissues. Phosphorylation was carried out as described in the legend to Fig. 2. After termination of the phosphorylation reaction with $NaDodSO_4$ (1% final concentration), samples were subjected to immunoprecipitation with 40 μl of 87-kDa protein antiserum, followed by $NaDodSO_4$/8% polyacrylamide gel electrophoresis. 87, 87-kDa protein. Positions of size markers are shown in kDa. (*B*) Autoradiogram showing phosphopeptide maps after limited proteolysis with *S. aureus* V8 protease of immunoprecipitated phosphorylated 87-kDa protein from various bovine tissues. Immunoprecipitation was carried out as in *A*. Phosphorylated 87-kDa protein, located by autoradiography, was excised and subjected to limited proteolysis during $NaDodSO_4$/15% polyacrylamide gel electrophoresis. 13 and 9, positions of the major and minor phosphorylated fragments derived from the 87-kDa protein. Positions of size markers are shown in kDa. Lane 1, cerebral cortex; lane 2, testis; lane 3, adrenal.

4.0–4.7) and, when subjected to limited proteolysis with *S. aureus* V8 protease, generated the same phosphopeptide map as the bovine protein. The 87-kDa protein has been identified by similar techniques in bovine vascular endothelial cells (20) and rat heart (unpublished results). Therefore, within a species the 87-kDa protein from each tissue was identical, although the concentration varied among the tissues, and the protein from rat tissues was of lower molecular mass than the protein from bovine tissues.

**Subcellular Distribution of the 87-kDa Protein.** The 87-kDa protein from bovine cerebral cortex was found to be distributed as follows: crude nuclear fraction ($P_1$), 20%; crude synaptosomal membrane ($P_2$-P), 40%; crude synaptosomal cytosol ($P_2$-S), 10%; crude microsomal fraction ($P_3$), 5%; and cytosol ($S_3$), 25% (Fig. 5*A*). This distribution is similar to that of synapsin I, a synaptic vesicle-associated protein, except that there is no synapsin I in the cytosol ($S_3$) (ref. 21 and not shown). The 87-kDa protein was not extracted from the crude synaptosomal membrane ($P_2$-P) with 0.5 M NaCl (Fig. 5*B*) but was almost completely extracted with nonionic detergents such as 1% NP-40 (Fig. 5*B*). In contrast, synapsin I, a peripheral membrane protein, behaved in an opposite manner, being extracted with 0.5 M NaCl but not with nonionic detergents (ref. 21 and not shown). Fractionation of a nonneuronal tissue (testis) showed that the 87-kDa protein partitioned roughly equally between the particulate and soluble fractions; again, the particulate protein was not extracted with 0.5 M NaCl but was extracted with 1% NP-40 (not shown). Further evidence of a membrane-bound fraction of 87-kDa protein was obtained by phase separation in Triton X-114 (22) using rat cerebellum; the 87-kDa protein partitioned between the detergent and water phases, whereas synapsin I was found in the water phase (not shown). Therefore, the 87-kDa protein behaved as an integral membrane protein and as a cytosolic protein.

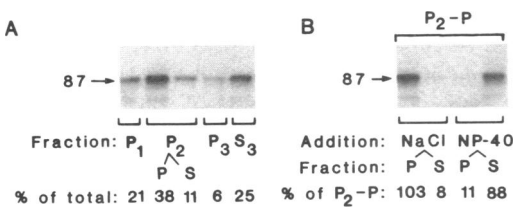


FIG. 5. (*A*) Immunoblot showing the distribution of 87-kDa protein in subcellular fractions from bovine cerebral cortex. Proteins in aliquots (10 μl) from the fractions were subjected to one-dimensional electrophoresis and immunoblotting as described in the legend to Fig. 2. Radioactivity in labeled proteins was quantitated by analysis of the blots with the AMBIS beta scanning system. $P_1$, crude nuclear fraction; $P_2$, crude synaptosomal fraction; $P_2$-P and $P_2$-S, particulate and soluble fractions, respectively, after lysis of $P_2$ followed by centrifugation at 35,000 × *g* for 30 min; $P_3$, crude microsomal fraction; $S_3$, cytosol. % of total, cpm in each fraction ×100 divided by the sum of cpm in all fractions shown. (*B*) Immunoblot showing extraction of 87-kDa protein from membrane by nonionic detergent but not by salt. Fractions were prepared by resuspension of the $P_2$-P fraction in buffer containing 0.5 M NaCl or 1% NP-40, incubation at 30°C for 1 hr, and centrifugation at 200,000 × *g* at 4°C for 30 min. Proteins in aliquots (10 μl) from the fractions were subjected to electrophoresis and immunoblotting as in *A*. NaCl-P and NaCl-S, particulate and soluble fractions, respectively, after resuspension of $P_2$-P in buffer containing 0.5 M NaCl followed by centrifugation. NP-40-P and NP-40-S, particulate and soluble fractions, respectively, after resuspension of $P_2$-P in buffer containing 1% NP-40 followed by centrifugation. % of $P_2$-P, cpm in each fraction ×100 divided by cpm in $P_2$-P. 87, Position of 87-kDa protein.

## DISCUSSION

The 87-kDa protein was identified previously as a major endogenous substrate for protein kinase C in broken cell preparations from rat brain (4, 5) and was found to be phosphorylated in intact nerve terminals (2). In the present study, the 87-kDa protein was identified in tissues from a number of species. Thus, the proteins from monkey, rat, and bovine brain and from a number of rat and bovine tissues were found to have similar properties: each was phosphorylated by exogenous protein kinase C and was of variable molecular mass depending on the gel system used; each was recognized by the 87-kDa antiserum; each was acidic and heterogeneous, with multiple phosphorylated spots of pI within the range 4.0–4.9; and each generated a characteristic phosphopeptide map upon limited digestion with V8 protease, with major and minor peptides having molecular masses of 13 kDa and 9 kDa, respectively. The 87-kDa protein was also identified in mouse and human brain and in *Torpedo californica* electric organ. The only detectable differences among the 87-kDa proteins were (*i*) that the relative concentration varied among the tissues, such that it was highest in brain, spleen, and lung, moderate in testis, pancreas, adrenal, kidney, and liver, and lowest in heart and skeletal muscle and (*ii*) that the protein from all rodent tissues had a molecular mass 4–7 kDa lower and was slightly more acidic than the proteins from tissues in the other species examined.

Similar results have been obtained recently by Blackshear *et al.* (23). They identified an acidic 80-kDa substrate for protein kinase C in heat-stable fractions from a number of rat tissues, which was most prominent in brain. This substrate had a lower molecular mass and was slightly more acidic in rat or mouse brain, fibroblasts, or fat pads than in bovine brain or fibroblasts or than in porcine, human, or rabbit brain. In addition, an antiserum to their bovine brain 80-kDa protein recognized purified bovine brain 87-kDa protein from our laboratory (P. J. Blackshear and L. Wen, personal communication).

Other laboratories have reported the phosphorylation of proteins of 80–83 kDa in response to activators of protein kinase C—e.g., in rat and mouse fibroblasts (23–26), cultured neuronal and glial cells (23), and rat superior cervical ganglion (A. Cahill and R. Perlman, personal communication). A comparison of the properties of these proteins makes it seem very likely that they are identical to the 87-kDa protein described in the present study.

Of particular interest is the subcellular distribution of the 87-kDa protein. In bovine cerebral cortex, most of the 87-kDa protein was found in the crude synaptosomal fraction and in the cytosol. When subfractionation of the synaptosomal fraction was carried out, about 80% of the synaptosomal 87-kDa protein was found in the synaptosomal particulate preparation; this was almost quantitatively extracted with nonionic detergents but not with NaCl. Further evidence for a membrane-bound fraction of the 87-kDa protein was obtained when the protein partitioned between the detergent and water phases in a Triton X-114 phase separation. Therefore, the 87-kDa protein behaves as an integral membrane protein and a soluble protein. A study of the relationship between the soluble and particulate forms of the protein requires further investigation.

The species, tissue, and subcellular distribution reported here for the 87-kDa protein follows very closely the reported localization for protein kinase C itself (6, 27–29), although the two proteins are distinct (7, 23). This localization pattern, coupled with the phosphorylation of the 87-kDa protein in response to activators of protein kinase C in intact synaptosomes (3) and in intact cells (23–26), or in response to depolarization-dependent $Ca^{2+}$ influx into intact synaptosomes (2), makes the 87-kDa substrate a likely mediator for certain functions of protein kinase C. Given the widespread tissue and species distribution of the protein reported here, such functions probably include cellular activities common to many cells. Further characterization of this 87-kDa protein, a major endogenous substrate for protein kinase C, will undoubtedly provide information about the actions of protein kinase C at the molecular level.

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