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Defining the Importance of Phosphatidylserine Synthase-1 (PSS1)

*UNEXPECTED VIABILITY OF PSS1-DEFICIENT MICE**

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Phosphatidylserine (PS) is a quantitatively minor, but physiologically important, phospholipid in mammalian cells. PS is synthesized by two distinct base-exchange enzymes, PS synthase-1 (PSS1) and PS synthase-2 (PSS2), that are encoded by different genes. PSS1 exchanges serine for choline of phosphatidylcholine, whereas PSS2 exchanges ethanolamine of phosphatidylethanolamine for serine. We previously generated mice lacking PSS2 (Bergo, M. O., Gavino, B. J., Steenbergen, R., Sturbois, B., Parlow, A. F., Sanan, D. A., Skarnes, W. C., Vance, J. E., and Young, S. G. (2002) *J. Biol. Chem.* 277, 47701–47708) and found that PSS2 is not required for mouse viability. We have now generated PSS1-deficient mice. In light of the markedly impaired survival of Chinese hamster ovary cells lacking PSS1 we were surprised that PSS1-deficient mice were viable, fertile, and had a normal life span. Total serine-exchange activity (contributed by PSS1 and PSS2) in tissues of *Pss1*$^{-/-}$ mice was reduced by up to 85%, but except in liver, the PS content was unaltered. Despite the presumed importance of PS in the nervous system, the rate of axonal extension of PSS1-deficient neurons was normal. Intercrosses of *Pss1*$^{-/-}$ mice and *Pss2*$^{-/-}$ mice yielded mice with three disrupted *Pss* alleles but no double knockout mice. In *Pss1*$^{-/-}$/*Pss2*$^{+/-}$ and *Pss1*$^{+/-}$/*Pss2*$^{-/-}$ mice, serine-exchange activity was reduced by 65–91%, and the tissue content of PS and phosphatidylethanolamine was also decreased. We conclude that (i) elimination of either PSS1 or PSS2, but not both, is compatible with mouse viability, (ii) mice can tolerate as little as 10% of normal total serine-exchange activity, and (iii) mice survive with significantly reduced PS and phosphatidylethanolamine content.

Phosphatidylserine (PS)[2] is a quantitatively minor phospholipid comprising 3–10% of total phospholipids of mammalian cell membranes. PS is synthesized by two distinct base-exchange enzymes, phosphatidylserine synthase-1 (PSS1) and phosphatidylserine synthase-2 (PSS2) (for a review, see Ref. 1). Each PS synthase is encoded by a different gene, but the enzymes share 32% amino acid identity (2). These two synthases are integral membrane proteins located primarily in mitochondria-associated membranes (MAM) (3). MAM comprise a specialized domain of the endoplasmic reticulum and are thought to mediate the import of PS into mitochondria via transient contact between MAM and mitochondrial outer membranes (4, 5). The PS synthases catalyze base-exchange reactions in which serine is exchanged for the polar head group of either phosphatidylcholine (PC) (PSS1 exchanges choline for serine) or phosphatidylethanolamine (PE) (PSS2 exchanges ethanolamine for serine).

Mutant Chinese hamster ovary (CHO) cells lacking PSS1 require supplementation with either ethanolamine (20 μM) or PS (10–50 μM) for survival (6, 7). In the absence of added ethanolamine, the mutant cells grow for two doublings, exhibit rounded morphology, and subsequently die, whereas the addition of ethanolamine supports growth (6). Normal growth of the PSS1-deficient cells was also restored by addition of PS to the culture medium. The ATP-dependent incorporation of choline into PC was undetectable in lysates of the mutant cells confirming that choline-exchange activity, contributed by PSS1, had been eliminated. The conversion of [3-^{3}H]serine to PS was reduced by ~50% in the PSS1-deficient CHO cells, and the amounts of PS and PE were 34 and 26% lower, respectively, than in parental CHO cells. Similar results were obtained in another mutant CHO cell line that also lacked PSS1 (7). Chemical mutagenesis of PSS1-deficient CHO cells generated a mutant cell line that contained only ~5% of the serine-exchange activity of parental CHO cells and had markedly reduced activities of both PSS1 and PSS2 (8). In the absence of PS supplementation, the PS content of these cells was 80% lower than that of parental CHO cells, and survival of the cells was markedly compromised (8). The conclusions from these studies with PS synthase-1-deficient CHO cells (6, 7) were that in CHO cells (i) PSS1 is essential for growth, and (ii) PSS1 is the major enzyme that produces PS. We, therefore, wished to determine the physiological importance of PSS1 in whole animals. We have previously generated PSS2-deficient mice (9). These mice are viable and appear outwardly normal. Some male *Pss2*$^{-/-}$ mice are infertile most likely because PSS2 is highly expressed in Sertoli cells of the testis. Serine-exchange activity

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[2] The abbreviations used are: PS, phosphatidylserine; CHO, Chinese hamster ovary; MAM, mitochondria-associated membranes; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PSS, phosphatidylserine synthase; PSD, PS decarboxylase; qPCR, quantitative PCR.

was reduced in all PSS2-deficient tissues examined and by >95% in testes. However, the phospholipid composition of all *Pss2*$^{-/-}$ tissues was normal. As compensation for the lack of PSS2 in hepatocytes, the choline-exchange activity of PSS1 was enhanced. Consequently the overall rate of PS synthesis in hepatocytes from the mice, as measured by the incorporation of [3-^{3}H]serine into PS, was unchanged by PSS2 deficiency (9, 10).

We have now generated PSS1-deficient mice to determine the importance of PSS1 in whole animals. The tissue distribution of PSS1 mRNA is different from that of PSS2 mRNA (9, 11, 12). Whereas PSS2 is highly expressed in Sertoli cells of the testis and Purkinje cells of the brain, PSS1 mRNA is more ubiquitously expressed with highest expression in liver, brain, kidney, and testis. In light of the impaired survival of PSS1-deficient CHO cells, we predicted that PSS1-deficient mice would exhibit severe abnormalities particularly in tissues that normally contain high levels of PSS1 activity. Contrary to our predictions, however, PSS1-deficient mice survive development and are viable. Although total serine-exchange activity in mouse tissues was markedly (by up to 85%) attenuated by PSS1 deficiency, the PS content of the tissues was only slightly, if at all, reduced. Even more surprisingly, intercrosses of *Pss1*$^{-/-}$ mice with *Pss2*$^{-/-}$ mice yielded viable mice with three disrupted *Pss* alleles. However, no double knock-out (*Pss1*$^{-/-}$/*Pss2*$^{-/-}$) mice were born. Thus, PSS1 is not essential for mouse development or survival, and only very low levels (<10% of normal) of serine-exchange activity are required for mouse viability. The reason why two mammalian PS synthases co-exist remains an enigma.

EXPERIMENTAL PROCEDURES

Generation and Genotyping of PSS1-deficent Mice—PSS1 knock-out mice (50% C57Bl/6, 50% 129OlaHsd) were generated with the assistance of inGenious Targeting Laboratories, Inc. (Stony Brook, NY). Briefly a 14.2-kb targeting vector was constructed with a 7.9-kb-long homology arm ending at the 5′-side of exon 3, a *Neo* cassette replacing 3.1 kb of the gene including exons 3 and 4, and a short homology arm extending 2 kb downstream of exon 4. The sequence of the targeting vector was confirmed by restriction analysis and sequencing and subcloned into a backbone vector. The construct was linearized and electroporated into 129SvEv embryonic stem cells. Homologous recombinants were selected by neomycin resistance and then identified by PCR analysis. The primers used were N1 (located in the *Neo* cassette) and A3 (located in intron 4/5 outside the region of the targeting vector sequence). These embryonic stem cell clones were injected into C57Bl/6 blastocysts and implanted into pseudopregnant female mice. Chimeric mice were crossed with C57Bl/6 mice, and heterozygotes were identified by genotyping of tail snips. Heterozygous mice were bred to yield *Pss1*$^{+/+}$, *Pss1*$^{+/-}$, and *Pss1*$^{-/-}$ mice. Mice were genotyped from DNA isolated from tail clippings using a DNEasy kit (Qiagen, Mississauga, Ontario, Canada). Genotyping was performed by PCR using the reverse primer 5′-AAG GCT TGC TTC CTA ACC AAG G-3′ with either the knock-out forward primer 5′-TGC GAG GCC AGA GGC CAC TTG TGT AGC-3′ or the wild-type forward primer 5′-GGC TGG ATC CCA ATC TTC GAT A-3′. The PCR product for the knock-out allele was 2.2 kb; the wild-type PCR product was also 2.2 kb (see Fig. 1*A*). PCR products were cloned and sequenced to confirm their identity. Knock-out genotype was confirmed with generic *Neo* primers (forward, 5′-GTT GTC ACT GAA GCG GGA AG-3′; reverse, 5′-AGC AAT ATC ACG GGT AGC CA-3′). Reverse transcription-PCR with primers corresponding to deleted exons 3 and 4 (forward, 5′-TCC ATT TAC TCG ACC TCA TCC-3′; reverse, 5′-TGA TGT CCG CTT CTC GTG TA-3′) gave no detectable band, confirming the absence of transcripts encompassing these exons. However, in homozygous knock-out animals, reverse transcription-PCR primers corresponding to sequences downstream of the knock-out cassette (forward, 5′-CCT TGT TGA TCC GTA GTT ATG GG-3′; reverse, 5′-TGC CCA GTG GTA AGT TCT CAT CTC-3′) revealed a band indicating the possible presence of a *Pss1* transcript. To determine the nature of this transcript, the message was amplified and cloned by reverse transcription-PCR using primers corresponding to the beginning and end of the coding sequence. Sequencing of this product showed the absence of exons 3 and 4 and a shift in the reading frame following these deleted regions.

Pss2 genotyping was performed with the following primers to give a 1.3-kb knock-out product: forward primer, 5′-TCA GCC AGG ACT CTA GAC ACA-3′; reverse primer, 5′-ATG GCT GGG ACC CTA AGA ATG-3′. The 1.1-kb wild-type product was amplified with the same forward primer and the reverse primer 5′-TGG AAT CTG GCA GTT CTC AGT-3′.

Mice were housed in a barrier facility at the University of Alberta with a 12-h light/dark cycle. Mice were weaned at 21 days of age after which they were fed a chow diet containing 4.5% fat (LabDiet 5001, Ralston Purina, St. Louis, MO). Adult mice (2–3 months old) were used for all experiments.

Real Time qPCR Measurement of mRNA Levels—Mouse tissues (~100 mg) were homogenized on ice with a Polytron (3 × 10-s bursts) in TRIzol reagent (Invitrogen). Total RNA was isolated according to the manufacturer's instructions. RNA quality was confirmed by electrophoresis on 1.5% formaldehyde-agarose gels and by measurement of the ratio of 28 S/18 S ribosomal RNA. Total RNA (5 μg) was digested with amplification-grade RNase-free DNase (Invitrogen) and then reverse transcribed in a 20-μl volume containing oligo(dT) and Superscript II (Invitrogen). Platinum SYBR Green qPCR Supermix-UDG (uracil-DNA glycosylase) was used to amplify genes in a Rotor-Gene RG-3000 thermocycler (Corbett Research, Mortlake, New South Wales, Australia). For all analyses, samples from at least three individual mice were used, and each sample was analyzed in triplicate. Data were analyzed using the standard curve method with normalization to cyclophilin A. Primers for cyclophilin A were: forward, 5′-TCC AAA GAC AGC AGA AAA CTT TCG-3′; and reverse, 5′-TCT TCT TGC TGG TCT TGC CAT TCC-3′. For PS decarboxylase the primers were: forward, 5′-TCT ACT GCC ACA CGC CAT T-3′; and reverse, 5′-AGC TTC CGT TCC CTG TAC TTC-3′. For PS synthase-2, the primers were: forward, 5′-ACT GTG CTG TTC ATC CTC ACC-3′; and reverse, 5′-AAA TGG CCC GTC TTT AGC-3′. For PS synthase-1, the primers were: forward, 5′-TCC ATT TAC TCG ACC TCA TCC-3′; and reverse, 5′-TGA TGT CCG CTT CTC GTG TA-3′.

Isolation of Subcellular Fractions—Fresh tissue samples from mice were homogenized with a Polytron in homogenization buffer containing 250 mM mannitol, 5 mM HEPES (pH 7.4), and 0.5 mM EGTA. Microsomes and mitochondria were isolated by sequential centrifugation as described previously (3, 4, 13). Briefly homogenates were centrifuged twice at 600 × *g* for 5 min to remove unbroken cells and nuclei, and then the supernatant was centrifuged at 10,300 × *g* for 10 min to yield a mitochondrial pellet that contained mitochondria with MAM. The postmitochondrial supernatant was centrifuged at 100,000 × *g* for 1 h at 4 °C to pellet microsomes. Microsomes and crude mitochondria were resuspended in homogenization buffer.

PS Synthase Activity—Serine-exchange activity was measured in aliquots (50–100 μg of protein) of freshly prepared homogenates, microsomes, and mitochondria using [3-^{3}H]serine (13). In some reactions, choline (50 mM) was included as a competitor for serine exchange to indicate activity contributed by PSS1. In addition, choline-exchange activity was directly measured using [*methyl*-^{14}C]choline in a reaction mixture (200 μl) that contained 100 μg of protein and 30–100 μCi of radiolabel in buffer containing 10 mM calcium chloride, 4 mM hydroxylamine, and 25 mM HEPES (pH 7.4). All enzymatic reactions were allowed to proceed for 20 min at 37 °C and were terminated by addition of chloroform/methanol (2:1, v/v). Water (1.5 ml) was added to each reaction tube, and samples were centrifuged to separate the phases. The lower phase was washed three times with 2 ml of methanol/water (1:1, v/v). The solvents were evaporated under a stream of nitrogen, and radioactivity was determined in phospholipid products. Enzymatic activity was calculated as nmol of PS or PC synthesized/h/mg of protein.

Phospholipid Content of Tissues and Subcellular Fractions—Mouse tissues were homogenized in a Polytron, and microsomes and crude mitochondria were isolated as described above. Lipids were extracted (14) and separated by thin-layer chromatography in the developing solvent system chloroform/methanol/acetic acid/formic acid/water (70:30:12:4:2). Phospholipids were identified by exposure of the plate to iodine vapor and comparison with authentic standards (Avanti Polar Lipids, Alabaster, AL). Bands corresponding to PC, PE, and PS were scraped from the plate, and the amount of each phospholipid (nmol/mg of protein) was determined by measurement of lipid phosphorus (15).

Metabolic Labeling of PS and PE in Primary Hepatocytes—Mice were anesthetized, and livers were perfused through the portal vein with Hanks' EGTA solution containing 10 μg/ml insulin until the liver was clear of blood. The superior and inferior vena cava were tied, and the perfusion was continued with Hanks' collagenase solution (100 units/ml) containing 10 μg/ml insulin until the liver softened (~3 min). The liver was removed, cut into pieces, transferred to Hanks' collagenase solution, and mixed until all clumps of tissue dissipated. The resulting hepatocytes were suspended in medium containing 10% fetal bovine serum and then plated on collagen-coated 60-mm dishes (2×10^6 cells/dish). Cell viability, typically >90% for both *Pss1*$^{+/+}$ and *Pss1*$^{-/-}$ hepatocytes, was estimated by trypan blue exclusion. After the hepatocytes had adhered to the dishes (2–3 h) the medium was removed and replaced with Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. Cells were used within 24 h of isolation.

For radiolabeling experiments, hepatocytes were washed twice with Dulbecco's modified Eagle's medium and then incubated in the same medium to which was added 3 μCi/ml [3-^{3}H]serine. For continuous labeling experiments, hepatocytes were incubated with [3-^{3}H]serine for the indicated times after which lipids were extracted (14). PS and PE were isolated by thin-layer chromatography in the developing solvent chloroform/methanol/acetic acid/formic acid/water (70:30:12:4:2). Because [3-^{3}H]serine is incorporated not only into the ethanolamine head group of PE but also into fatty acyl chains and the glycerol backbone (16), PE was hydrolyzed with phospholipase C from *Bacillus cereus* to release the ethanolamine moiety (10, 17). For pulse-chase experiments, hepatocytes were incubated with [3-^{3}H]serine for 1 h after which radioactive medium was replaced with unlabeled Dulbecco's modified Eagle's medium for the indicated times.

Axonal Extension of Mouse Sympathetic Neurons—Sympathetic neurons were isolated from the superior cervical ganglia of *Pss1*$^{+/+}$ and *Pss1*$^{-/-}$ mice and plated in three-compartment culture dishes as described previously (18). The neurons were cultured in medium containing 20 ng/ml nerve growth factor in the center compartment that contained cell bodies with proximal axons and 50 ng/ml nerve growth factor in the two side compartments that contained distal axons alone. After 7 days, distal axons were removed (axotomized) from side compartments with a jet of sterile water delivered through a 22-gauge needle. The length of the axons was measured in 36 tracks/dish from at least four dishes (19). Axon length is given as average ± S.E. from at least three independent preparations of neurons from *Pss1*$^{+/+}$ and *Pss1*$^{-/-}$ mice.

Other Methods—The protein content of samples was determined by the BCA method (Pierce) with bovine serum albumin as standard.

RESULTS

PSS1-deficient Mice Are Viable—To determine the importance of PSS1 in whole animals we generated PSS1-deficient mice. Male and female offspring of the three *Pss1* genotypes were produced from *Pss1*$^{+/-}$ breeding pairs in approximately the predicted Mendelian distribution of 1:2:1 (*Pss1*$^{+/+}$/*Pss1*$^{+/-}$/*Pss1*$^{-/-}$) as demonstrated by PCR genotyping (Fig. 1*A*). No *Pss1* mRNA was detected in brain, liver, and heart from *Pss1*$^{-/-}$ mice; the level of *Pss1* mRNA in *Pss1*$^{+/-}$ mice was ~50% of that in *Pss1*$^{+/+}$ mice (Fig. 1*B*). Male and female *Pss1*$^{+/-}$ and *Pss1*$^{-/-}$ mice are outwardly normal and have a normal lifespan, and both males and females are fertile. The rate of weight gain of male *Pss1*$^{-/-}$ and *Pss1*$^{+/+}$ mice fed a chow diet over 20 weeks was indistinguishable (not shown). Similar results were obtained for female *Pss1*$^{+/+}$ and *Pss1*$^{-/-}$ mice (not shown).

Pss2 mRNA Is Not Compensatorily Increased in Pss1$^{-/-}$ *Mice*—To determine whether expression of *Pss2* mRNA was increased as compensation for the lack of PSS1, brain, liver, and heart were removed from adult male *Pss1*$^{+/+}$, *Pss1*$^{+/-}$, and *Pss1*$^{-/-}$ mice, and *Pss2* mRNA levels were assessed by real time qPCR analysis relative to the level of cyclophilin A mRNA. As shown in Fig. 2*A*, the amount of *Pss2* mRNA in these tissues was

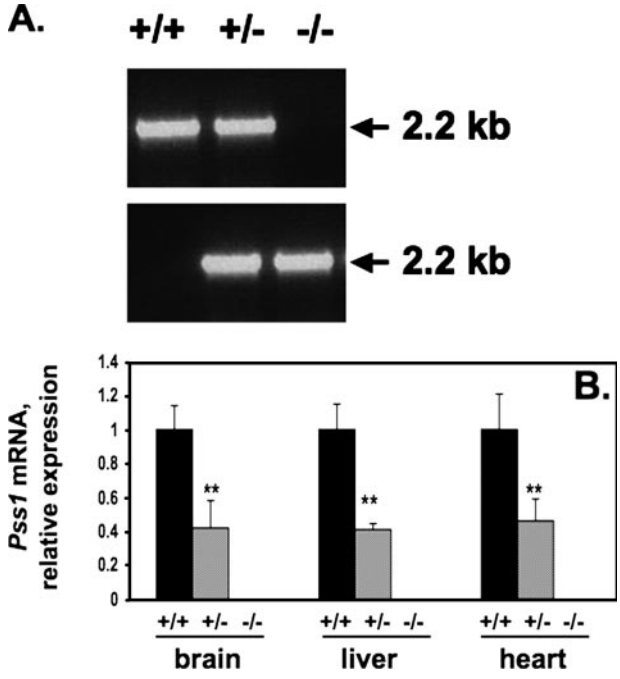

FIGURE 1. **Genotyping and *Pss1* mRNA levels of $Pss1^{+/+}$, $Pss1^{+/-}$, and $Pss1^{-/-}$ mice.** *A*, tail clippings from mice were genotyped by PCR analysis. The size of the PCR product for the wild-type allele (*top panel*) (2.2 kb) is the same as for the disrupted allele (2.2 kb) (*bottom panel*). *B*, brains, livers, and hearts were removed from adult $Pss1^{+/+}$, $Pss1^{+/-}$, and $Pss1^{-/-}$ mice. *Pss1* mRNA levels were determined relative to cyclophilin mRNA by real time qPCR. Data are averages ± S.D. from at least three mice, each with triplicate analyses. *Black bars*, $Pss1^{+/+}$; *hatched bars*, $Pss1^{+/-}$. *Pss1* mRNA was undetectable in $Pss1^{-/-}$ mice. **, $p < 0.05$.

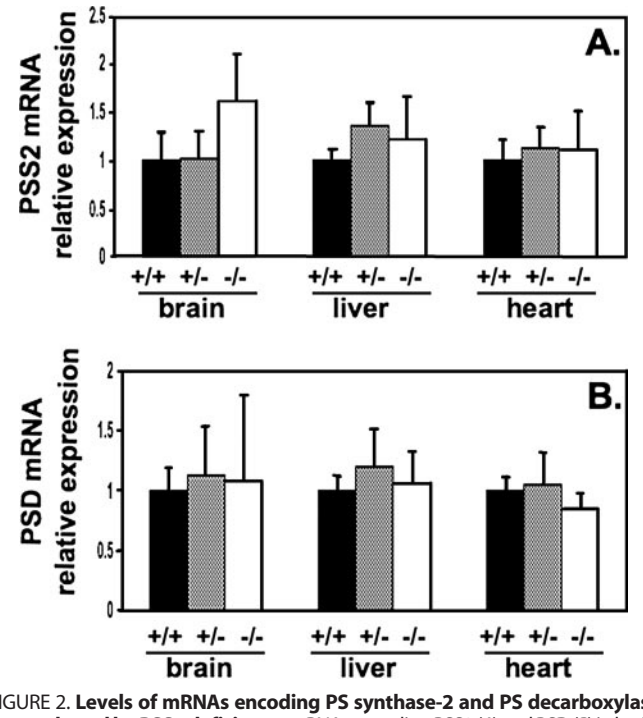

FIGURE 2. **Levels of mRNAs encoding PS synthase-2 and PS decarboxylase are unaltered by PSS1 deficiency.** mRNAs encoding PSS2 (*A*) and PSD (*B*) in brain, liver, and heart from adult male mice were quantified relative to cyclophilin mRNA by real time qPCR. Data are averages ± S.D. from at least three mice, each with triplicate measurements. *Black bars*, $Pss1^{+/+}$; *hatched bars*, $Pss1^{+/-}$; *white bars*, $Pss1^{-/-}$.

independent of *Pss1* genotype. Similar results were obtained for brain, liver, and heart from female mice (not shown). Another important enzyme that is involved in PS metabolism is the mitochondrial enzyme PS decarboxylase (PSD) that converts PS to PE (20, 21). The large majority of PE in mitochondria is produced from PS via PSD (5). Thus, we considered the possibility that if the supply of PS were reduced by elimination of PSS1 then PSD expression might be increased as a compensatory mechanism for maintaining normal levels of mitochondrial PE. Fig. 2*B* shows, however, that this was not the case and that PSD mRNA was unchanged by PSS1 deficiency.

PS Synthase Activity Is Reduced in Pss1 Knock-out Mice—Pss1 mRNA is highly expressed in brain, liver, and heart of $Pss1^{+/+}$ mice but is undetectable in the corresponding tissues from $Pss1^{-/-}$ mice (Fig. 1*B*). We were unable to determine directly whether PSS1 protein was absent from *Pss1* knock-out mice because PSS1-specific antibodies were not available. However, we compared the total serine-exchange activity, representing the combined activities of PSS1 and PSS2, in homogenates of brain, liver, heart, and testis of mice of the three *Pss1* genotypes (Fig. 3). In all experiments, PS synthase activity was measured in freshly isolated tissue homogenates and membrane fractions because this enzymatic activity is labile and decreases significantly upon sample storage.[3] In homogenates of brain (Fig. 3*A*), liver (Fig. 3*B*), and heart (Fig. 3*C*) of $Pss1^{-/-}$ mice the total serine-exchange activity was markedly lower (by 62% in brain, by 67% in liver, and by 55% in heart) than in the corresponding $Pss1^{+/+}$ tissues. In contrast, serine-exchange activity in the testis was unaltered by PSS1 deficiency (Fig. 3*D*), consistent with our previous observation that the vast majority of serine-exchange activity in testis, which is severalfold higher than in other tissues, is contributed by PSS2 (9).

Serine-exchange activity was also measured in microsomal fractions isolated from homogenates of brain, liver, and heart of the mice (Fig. 3). In general, these activities mirrored those in tissue homogenates. Microsomal serine-exchange activity was significantly lower (by 52% in brain, by 85% in liver, and by 50% in heart) in $Pss1^{-/-}$ mice compared with $Pss1^{+/+}$ mice. A significant portion of the serine-exchange activity in microsomes is likely contributed by MAM (3, 22). MAM are highly enriched in PSS1 and PSS2 activities (3) and represent a subfraction of the endoplasmic reticulum that is thought to participate in the import of newly synthesized PS into mitochondria (5). In addition, serine-exchange activity was measured in crude mitochondrial fractions that contain both mitochondria and MAM; purified mitochondria are devoid of significant amounts of PS synthase activity (4, 23). Thus, serine-exchange activity in the crude mitochondrial fraction can be attributed to MAM. Serine-exchange activity in mitochondria/MAM was lower in $Pss1^{+/-}$ livers and hearts than in the corresponding $Pss1^{+/+}$ tissues and even lower in mitochondrial fractions from $Pss1^{-/-}$ tissues (Fig. 3). Therefore, total serine-exchange activity in brain, liver, and heart is significantly reduced by PSS1 deficiency.

PSS1 catalyzes the exchange of serine for the choline head group of PC, whereas PSS2 catalyzes the exchange of serine for ethanolamine but not choline (8). The activities of PSS1 and PSS2 can, therefore, be distinguished by comparing *in vitro* ser-

[3] J. E. Vance, unpublished observations.

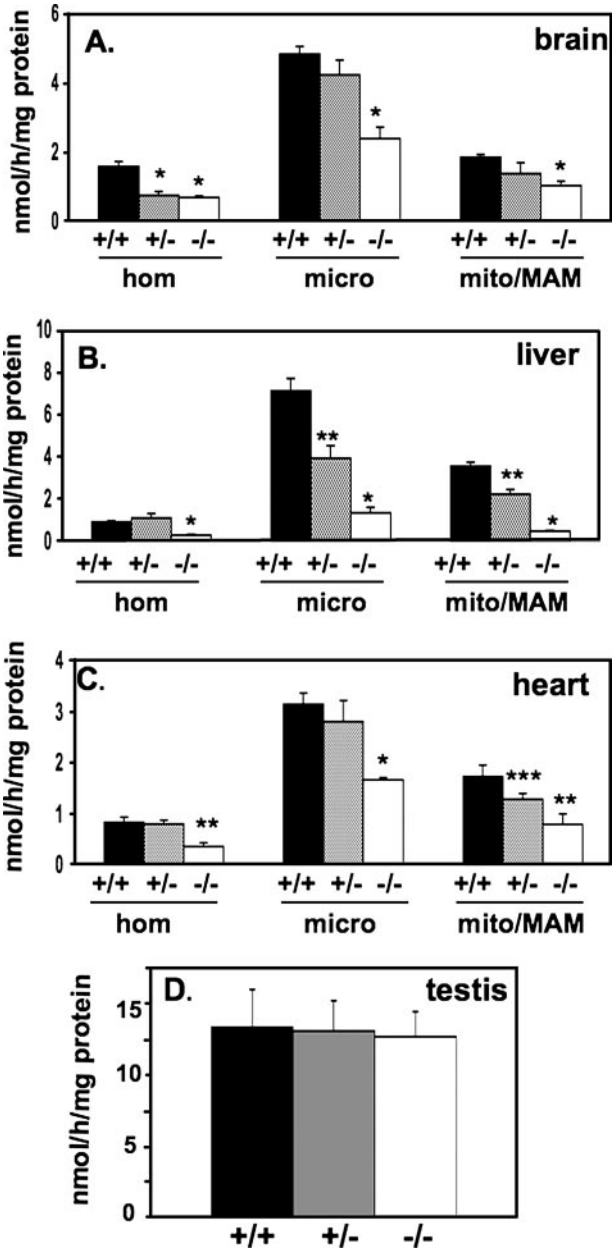

FIGURE 3. **Serine-exchange activity in tissues from *Pss1*$^{+/+}$, *Pss1*$^{+/-}$, and *Pss1*$^{-/-}$ mice.** Brains (*A*), livers (*B*), and hearts (*C*) from male adult mice of the three *Pss1* genotypes were homogenized after which microsomes and crude mitochondria were isolated. The mitochondrial fraction contains mitochondria as well as MAM. Serine-exchange activity was measured *in vitro* in homogenates (*hom*), microsomes (*micro*), and crude mitochondria (*mito/MAM*) using [3-^{3}H]serine. Total serine-exchange activity reflects activity contributed by PSS1 and PSS2. *D*, serine-exchange activity was measured in homogenates of testes from the same mice. All data are averages ± S.D. from at least three mice, each with triplicate measurements. *Black bars*, *Pss1*$^{+/+}$; *hatched bars*, *Pss1*$^{+/-}$; *white bars*, *Pss1*$^{-/-}$. *, $p < 0.001$; **, $p < 0.01$; ***, $p < 0.05$ compared with *Pss1*$^{+/+}$ mice.

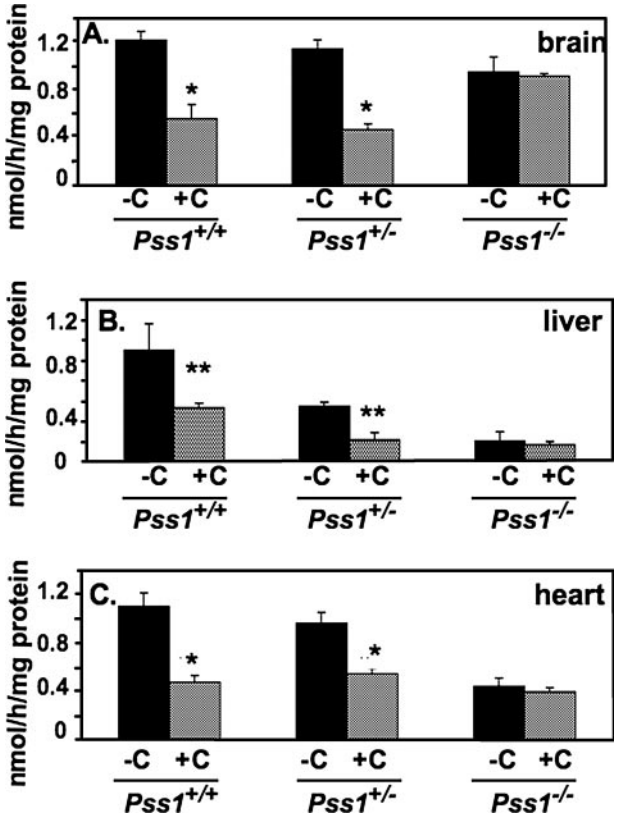

FIGURE 4. **PS synthase-1 activity in tissues from *Pss1*$^{+/+}$, *Pss1*$^{+/-}$, and *Pss1*$^{-/-}$ mice.** Brains (*A*), livers (*B*), and hearts (*C*) from adult male mice of the three *Pss1* genotypes were homogenized, and serine-exchange activity was measured in homogenates using [3-^{3}H]serine in the presence (+*C*, *hatched bars*) or absence (−*C*, *black bars*) of 50 mM choline. Total serine-exchange activity is contributed by both PSS1 and PSS2, whereas serine-exchange activity remaining in the presence of choline represents PSS2 activity. Data are averages ± S.D. from at least three mice, each with triplicate analyses. *, $p < 0.001$; **, $p < 0.01$.

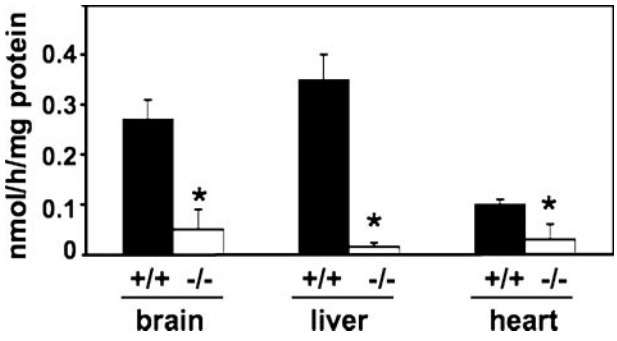

FIGURE 5. **Choline-exchange activity in tissues from *Pss1*$^{+/+}$, *Pss1*$^{+/-}$, and *Pss1*$^{-/-}$ mice.** Brains, livers, and hearts were removed from adult male *Pss1*$^{+/+}$ and *Pss1*$^{-/-}$ mice and homogenized. Choline-exchange activity (contributed by PSS1) was measured in homogenates with [*methyl*-^{14}C]choline and is given as radioactivity incorporated into phosphatidylcholine. Data are averages ± S.D. from at least three mice, each with triplicate analyses. *Black bars*, *Pss1*$^{+/+}$; *white bars*, *Pss1*$^{-/-}$. *, $p < 0.001$.

ine-exchange activities in the absence and presence of choline because choline competes with [^{3}H]serine for PSS1 but not PSS2. Consequently the contribution of PSS1 to total serine-exchange activity can be assessed. The presence of 50 mM choline in the enzymatic assays reduced serine-exchange activity in homogenates of *Pss1*$^{+/+}$ brain (Fig. 4*A*), liver (Fig. 4*B*), and heart (Fig. 4*C*) by 53–73% and in homogenates of *Pss1*$^{+/-}$ tissues by 38–60%. In contrast and as expected for cells lacking PSS1, choline did not compete with [3-^{3}H]serine in measurements of serine-exchange activity of tissues from *Pss1*$^{-/-}$ mice (Fig. 4). The choline-exchange activity of PSS1 was also assessed by measurement of the direct incorporation of [^{14}C]choline into PC via base exchange (3). Fig. 5 shows that in brain, liver, and heart from *Pss1*$^{-/-}$ mice the choline-exchange activity was 75–94% lower than in the corresponding *Pss1*$^{+/+}$

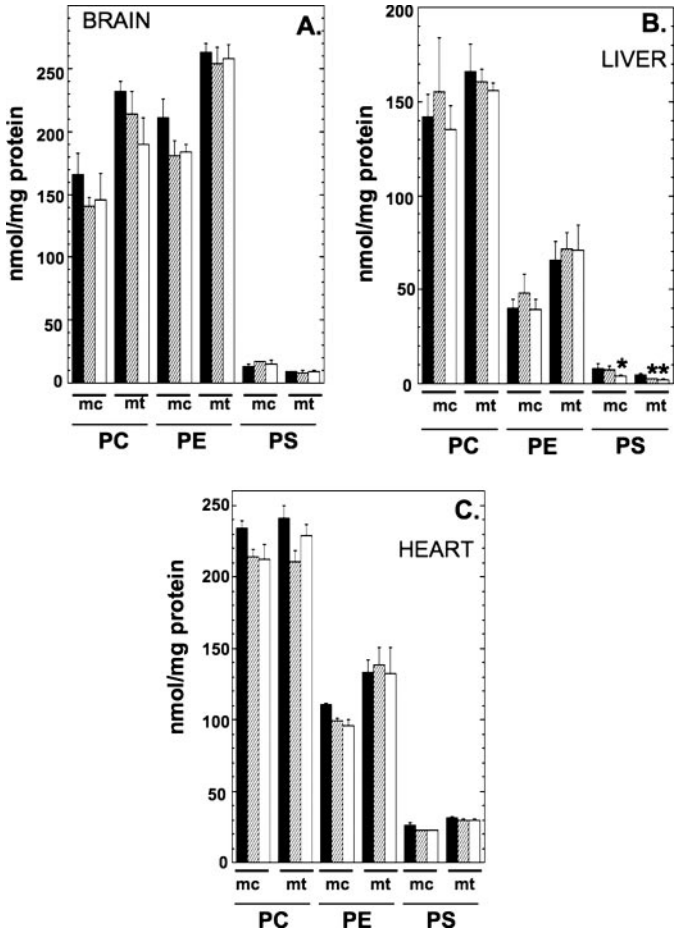

FIGURE 6. **Phospholipid content of tissues from *Pss1*$^{+/+}$, *Pss1*$^{+/-}$, and *Pss1*$^{-/-}$ mice.** Brains (*A*), livers (*B*), and hearts (*C*) from adult male mice of the three *Pss1* genotypes were homogenized. Microsomes (*mc*) and mitochondria (*mt*) were isolated, and lipids were extracted. PC, PE, and PS were isolated by thin-layer chromatography, and amounts were quantified by phosphorus analysis. *Black bars*, *Pss1*$^{+/+}$; *hatched bars*, *Pss1*$^{+/-}$; *white bars*, *Pss1*$^{-/-}$. Data are averages ± S.D. from at least three mice, each with triplicate analyses. *, $p < 0.01$.

tissues. These combined observations confirm that PSS1 was eliminated from the *Pss1*$^{-/-}$ mice and that serine- and choline-exchange activities in tissues of the mice were significantly reduced.

PSS1 Deficiency Only Modestly Alters Phospholipid Composition—Because PS synthase activity was greatly reduced in tissues from *Pss1*$^{-/-}$ mice we determined whether or not the phospholipid content of the brain (Fig. 6*A*), liver (Fig. 6*B*), and heart (Fig. 6*C*) was altered in *Pss1*$^{+/-}$ and *Pss1*$^{-/-}$ mice. Microsomes and mitochondria were isolated from homogenates of tissues from *Pss1*$^{+/+}$, *Pss1*$^{+/-}$, and *Pss1*$^{-/-}$ mice, and the amounts of PC, PE, and PS were measured. Because mitochondrial PE is generated primarily in mitochondria from PS via PSD (5), we hypothesized that a decrease in PS synthesis might reduce the amount of mitochondrial PE. In brain (Fig. 6*A*) and heart (Fig. 6*C*) the PC, PE, and PS content of microsomes and mitochondria was not significantly different among the three *Pss1* genotypes. In contrast, in livers, the mass of PS in microsomes and mitochondria was 48% (4.3 ± 0.2 *versus* 8.2 ± 2.3 nmol/mg of protein) and 60% (1.9 ± 0.6 *versus* 4.7 ± 0.7 nmol/mg of protein), respectively, lower in *Pss1*$^{-/-}$ mice than in *Pss1*$^{+/+}$ mice (Fig. 6*B*). The amounts of PC and PE in liver microsomes and mitochondria were independent of *Pss1* genotype. Although the PS content of livers of *Pss1*$^{-/-}$ mice was reduced by ~50%, the livers appeared grossly normal upon visual inspection. Despite profound reductions in serine- and choline-exchange activities in heart and brain of PSS1 knock-out mice, the PS content was not reduced.

Altered PS Metabolism in Pss1$^{-/-}$ *Hepatocytes*—The observations reported above are consistent with the view that phospholipid homeostasis in mammalian cells is tightly regulated and that when PS synthesis is attenuated cells implement compensatory mechanisms in an attempt to maintain a constant phospholipid composition. This type of regulation was observed previously in tissues of PSS2-deficient mice in which the amount of PS was unaltered in the face of large reductions in total serine-exchange activity (9, 10). To determine whether compensatory changes in PS metabolism occurred in response to PSS1 deficiency we cultured primary hepatocytes from *Pss1*$^{+/+}$ and *Pss1*$^{-/-}$ mice. The viability of *Pss1*$^{-/-}$ and *Pss1*$^{+/+}$ hepatocytes, as estimated by trypan blue exclusion, was indistinguishable (>90% 16 h after plating). We examined the rate of synthesis of PS and PS-derived PE in radiolabeling experiments using [3-^{3}H]serine as a tracer. Because [3-^{3}H]serine that is incorporated into the ethanolamine head group of PE must have been derived from newly made PS, we included both the radiolabel in the ethanolamine moiety of PE and the radiolabel in PS as an indication of the rate of PS synthesis (Fig. 7*A*). Furthermore [^{3}H]serine is metabolically incorporated not only into the ethanolamine head group of PE via PSD but also into the fatty acyl chains and the glycerol backbone of PE (17). We, therefore, hydrolyzed the isolated PE with phospholipase C to release the ethanolamine moiety and determined the amount of radioactivity in ethanolamine as an indication of the conversion of PS to PE. Very little [3-^{3}H]serine is incorporated into the choline moiety of PC (via PS decarboxylation followed by methylation of PE to PC) during a 4-h time period in rodent hepatocytes (~1% of that in the ethanolamine head group of PE) (17). The incorporation of [3-^{3}H]serine into PS, combined with radiolabel in the ethanolamine portion of PE, over a 2-h time period was reduced by 20–40% by PSS1 deficiency (Fig. 7*A*). This observation is consistent with (i) the reduced mass of PS in livers from PSS1-deficient mice (Fig. 6*B*), (ii) the 85% reduction in total serine-exchange activity observed in *in vitro* enzymatic assays of liver microsomes (Fig. 3*B*), and (iii) the lack of up-regulation of *Pss2* mRNA expression in PSS1-deficient tissues (Fig. 2*A*).

The rate of incorporation of [3-^{3}H]serine into the ethanolamine head group of PE via PSD was unaffected by PSS1 deficiency (Fig. 7*B*). Because both the mass and radioactivity of PS, the precursor of [^{3}H]PE, were reduced to a similar extent in *Pss1*$^{-/-}$ livers/hepatocytes, the specific radioactivity (dpm/nmol) of the PS precursor of PE is expected to be similar in *Pss1*$^{+/+}$ and *Pss1*$^{-/-}$ hepatocytes. Thus, we conclude that the rate of PS decarboxylation is not significantly altered by PSS1 deficiency. The degradation of newly synthesized PS (which includes both the conversion of PS to PE and the degradation of PS via unidentified phospholipases) was examined in pulse-chase experiments. *Pss1*$^{+/+}$ and *Pss1*$^{-/-}$ hepatocytes were

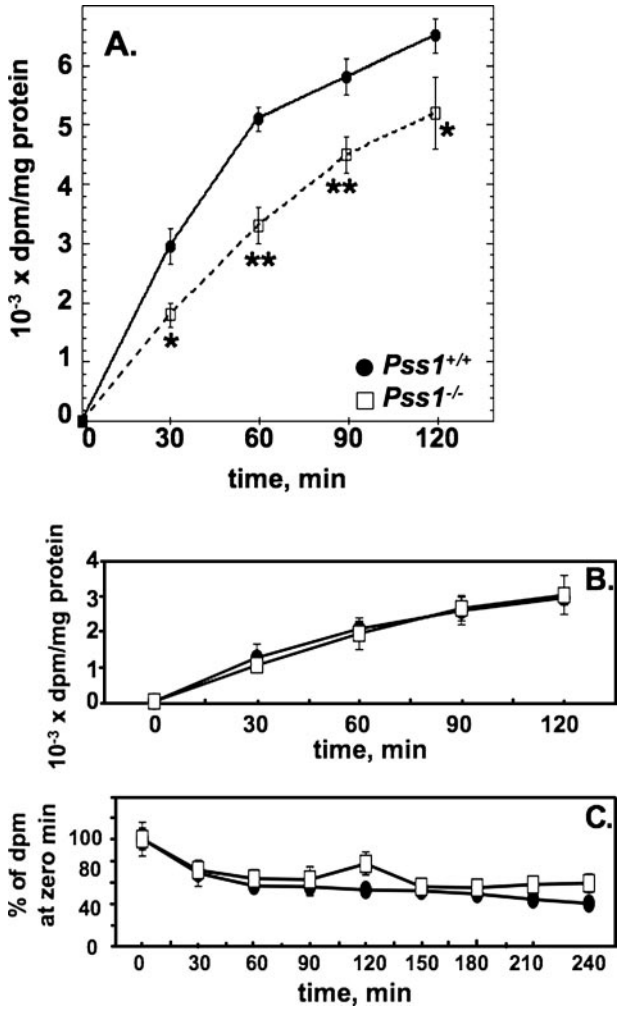

FIGURE 7. **Synthesis and degradation of PS in hepatocytes from *Pss1*$^{+/+}$ and *Pss1*$^{-/-}$ mice.** Primary hepatocytes were isolated from adult male *Pss1*$^{+/+}$ (*filled symbols*) and *Pss1*$^{-/-}$ (*open symbols*) mice. *A* and *B*, hepatocytes were incubated for up to 120 min with [3-^{3}H]serine. Lipids were extracted, and PS and PE were isolated by thin-layer chromatography. Radioactivity in PS plus the ethanolamine moiety of PE (*A*) and radioactivity in the ethanolamine moiety of PE alone (*B*) were determined. Data are averages ± S.D. of triplicate analyses from a representative experiment. Similar results were obtained in three additional experiments. *, $p < 0.05$; **, $p < 0.01$. *C*, hepatocytes were incubated with [3-^{3}H]serine for 1 h (time = 0 min) after which radioactive medium was replaced with medium containing unlabeled serine. At the indicated chase times, radioactivity was measured in PS. The rate of degradation of PS is indicated by dpm in PS/mg of protein as a percentage of radioactivity at the start of the chase period (0 min). *Solid symbols*, *Pss1*$^{+/+}$; *open symbols*, *Pss1*$^{-/-}$. Data are averages ± S.D. from a representative experiment; similar results were obtained in three additional experiments.

labeled with [3-^{3}H]serine for 1 h. The radiolabeled medium was subsequently removed, and the rate of decline in radioactivity in PS was monitored over the next 4 h. The rate of decay of [^{3}H]PS was not significantly different between *Pss1*$^{+/+}$ and *Pss1*$^{-/-}$ hepatocytes (Fig. 7*C*), indicating that PS degradation in hepatocytes is unaffected by PSS1 deficiency.

The data from cultured hepatocytes imply that the rate of PS synthesis is significantly reduced by PSS1 deficiency, whereas PS degradation is unaffected. A decrease in the rate of PS synthesis without an accompanying reduction in the rate of PS degradation is consistent with the observed ~50% lower levels of PS in livers of *Pss1*$^{-/-}$ compared with *Pss1*$^{+/+}$ mice (Fig. 6*B*).

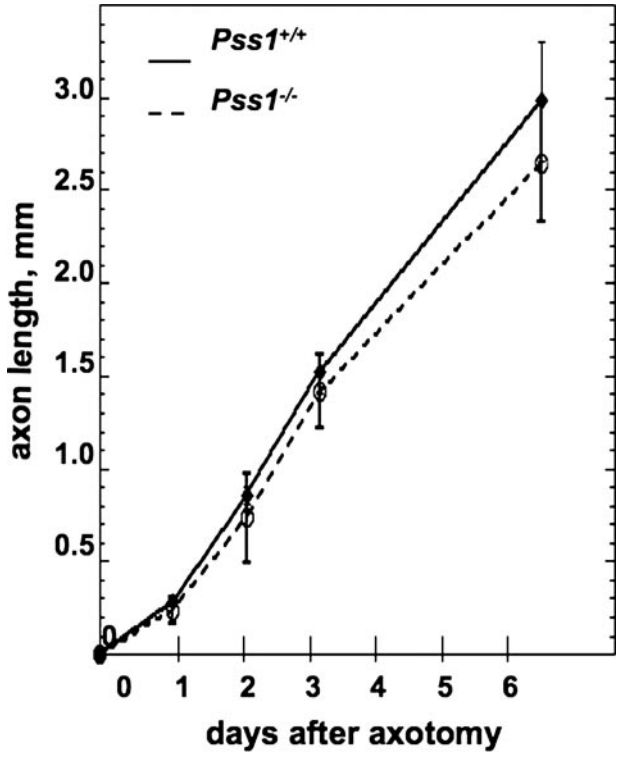

FIGURE 8. **Axonal extension of sympathetic neurons from *Pss1*$^{+/+}$ and *Pss1*$^{-/-}$ mice.** Sympathetic neurons were isolated from *Pss1*$^{+/+}$ and *Pss1*$^{-/-}$ mice and cultured in compartmented dishes for 7 days. At this time, distal axons were removed and allowed to regenerate in the presence of 50 ng/ml nerve growth factor. Axon length was measured over a 6-day period in 36 tracks/dish in each of at least four dishes. Data for axon length (mm) are averages ± S.E. from at least three independent preparations of neurons from *Pss1*$^{+/+}$ (*solid line*) and *Pss1*$^{-/-}$ (*dashed line*) mice.

Axonal Extension of Pss1$^{+/+}$ *and Pss1*$^{-/-}$ *Sympathetic Neurons*—PS is enriched in the brain compared with other tissues. The molecular species of PS in brain and particularly in the retina (24–26) are unusual because the majority of PS in this tissue contains one or two docosahexaenoic acid (22:6*n*-3) acyl chains. PS that contains docosahexaenoic acid appears to be essential for optimal neuronal and visual functions (for a review, see Ref. 27). We, therefore, hypothesized that a PSS1 deficiency might impair some key neuronal functions, such as axonal elongation. Sympathetic neurons from *Pss1*$^{+/+}$ and *Pss1*$^{-/-}$ mice were cultured in compartmented culture dishes (28), and the rate of axonal extension was measured. Over a 6-day period, the rate of axon growth was not significantly different between *Pss1*$^{+/+}$ and *Pss1*$^{-/-}$ neurons (Fig. 8).

Pss1$^{-/-}$*/Pss2*$^{+/-}$ *Mice and Pss1*$^{+/-}$*/Pss2*$^{-/-}$ *Mice Are Viable*—As is the case for PSS2-deficient mice (9), PSS1-deficient mice appear outwardly normal. Because PSS1 deficiency only modestly decreased the PS content of tissues, we attempted to reduce PS synthase activity and PS levels further by crossing *Pss1*$^{-/-}$ mice with *Pss2*$^{-/-}$ mice to generate *Pss1*$^{-/-}$/*Pss2*$^{+/-}$, *Pss1*$^{+/-}$/*Pss2*$^{-/-}$, and perhaps *Pss1*$^{-/-}$/*Pss2*$^{-/-}$ double knock-out mice. *Pss1*$^{-/-}$/*Pss2*$^{+/-}$ mice and *Pss1*$^{+/-}$/*Pss2*$^{-/-}$ mice are both viable. In contrast, of a total of >100 offspring, no double knock-out (*Pss1*$^{-/-}$/*Pss2*$^{-/-}$) offspring were born. Total serine-exchange activity (PSS1 and PSS2 activities combined) was measured in brain, liver, and heart of *Pss1*$^{-/-}$/*Pss2*$^{+/-}$ mice and *Pss1*$^{+/-}$/*Pss2*$^{-/-}$ mice and com-

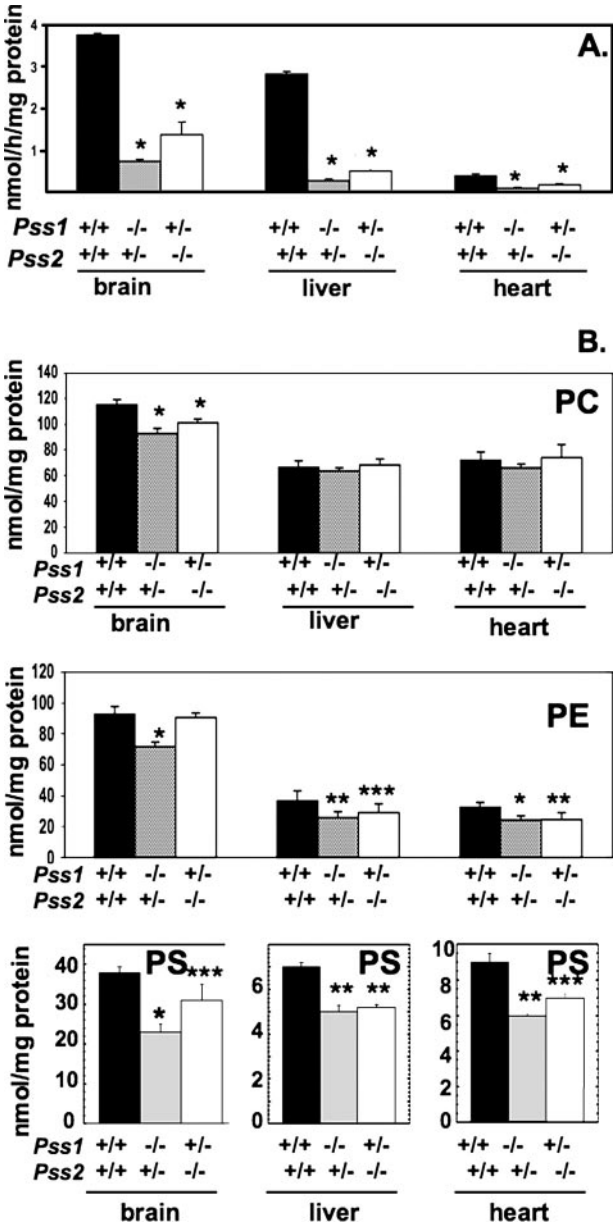

FIGURE 9. **Serine-exchange activity and phospholipid content of tissues from mice with three inactivated *Pss* alleles.** *Pss1*$^{-/-}$ mice were bred with *Pss2*$^{-/-}$ mice. No *Pss1*$^{-/-}$/*Pss2*$^{-/-}$ (double knock-out) offspring survived, whereas mice with three inactivated *Pss* alleles (*Pss1*$^{-/-}$/*Pss2*$^{+/-}$ and *Pss1*$^{+/-}$/*Pss2*$^{-/-}$ mice) were viable. Brains, livers, and hearts were removed from male *Pss1*$^{+/+}$/*Pss2*$^{+/+}$ (*black bars*), *Pss1*$^{-/-}$/*Pss2*$^{+/-}$ (*hatched bars*), and *Pss1*$^{+/-}$/*Pss2*$^{-/-}$ (*white bars*) mice, and tissues were homogenized. *A*, serine-exchange activity was measured in tissue homogenates. *B*, lipids were extracted from tissue homogenates. PC, PE, and PS were isolated by thin-layer chromatography and quantified by phosphorus analysis. *Black bars*, *Pss1*$^{+/+}$/*Pss2*$^{+/+}$; *hatched bars*, *Pss1*$^{-/-}$/*Pss2*$^{+/-}$; *white bars*, *Pss1*$^{+/-}$/*Pss2*$^{-/-}$. All data are averages ± S.D. of triplicate measurements from at least three mice of each genotype. *, $p < 0.001$; **, $p < 0.01$; ***, $p < 0.05$ compared with *Pss1*$^{+/+}$/*Pss2*$^{+/+}$ mice.

pared with that of *Pss1*$^{+/+}$/*Pss2*$^{+/-}$ mice (Fig. 9*A*). Inactivation of three PS synthase alleles reduced serine-exchange activity by 65–91%. In all three tissues examined, the elimination of PSS1 in *Pss2*$^{+/-}$ mice reduced total PS synthase activity to a greater extent (by 80% in brain, 91% in liver, and 89% in heart) than did elimination of PSS2 in *Pss1*$^{+/-}$ mice (by 66% in brain, 79% in liver, and 56% in heart).

To determine the biochemical impact of the profound reduction in PS-synthesizing capacity, the phospholipid composition of tissue homogenates from the *Pss1*$^{-/-}$/*Pss2*$^{+/-}$ mice and *Pss1*$^{+/-}$/*Pss2*$^{-/-}$ mice was determined (Fig. 9*B*). The PS content of *Pss1*$^{-/-}$/*Pss2*$^{+/-}$ mouse tissues was significantly lower (by 40% in brain, by 29% in liver, and by 33% in heart) than in *Pss1*$^{+/+}$/*Pss2*$^{+/+}$ tissues. A similar, but not so pronounced, reduction in PS content of tissues also occurred in *Pss1*$^{+/-}$/*Pss2*$^{-/-}$ mice (Fig. 9*B*). Furthermore in tissue homogenates from *Pss1*$^{-/-}$/*Pss2*$^{+/-}$ mice the PE content was lower (by 23% in brain, by 29% in liver, and by 25% in heart) than in *Pss1*$^{+/+}$/*Pss2*$^{+/+}$ mice; the PE content of tissues from *Pss1*$^{+/-}$/*Pss2*$^{-/-}$ mice was similarly reduced. In tissues from mice in which three *Pss* alleles were inactivated the PC content was reduced only slightly if at all. Remarkably, however, despite the striking decrease in both PS biosynthetic capacity and the PS and PE content of tissues, the *Pss1*$^{-/-}$/*Pss2*$^{+/-}$ and *Pss1*$^{+/-}$/*Pss2*$^{-/-}$ mice are viable. Preliminary experiments indicate that the life span and weight gain of *Pss1*$^{-/-}$/*Pss2*$^{+/-}$, *Pss1*$^{+/-}$/*Pss2*$^{-/-}$, and *Pss1*$^{+/+}$/*Pss2*$^{+/+}$ mice are similar.

DISCUSSION

We have demonstrated that PS synthesis via PSS1 is not essential for mouse development or survival. Despite a large (>50%) reduction in total serine-exchange activity and complete elimination of choline-exchange activity in PSS1-deficient mice, the expression of the mRNA encoding PSS2, which catalyzes the alternative pathway for PS synthesis, was not up-regulated. Nor were the levels of PS and PE markedly altered by PSS1 deficiency except in the liver, a prominent site of *Pss1* mRNA expression (11), where the mass of PS in microsomes and mitochondria was decreased by ~50%. The reduced level of PS in livers of *Pss1*$^{-/-}$ mice is consistent with results from radiolabeling experiments in primary hepatocytes in which the rate of PS synthesis, as measured by the incorporation of [3-^{3}H]serine into PS, was reduced by ~40%, whereas the rate of PS degradation was unchanged. Two potential explanations for why the mass of PS in brain and heart was not reduced by PSS1 deficiency in the face of such a large reduction in serine-exchange activity are the following. First, compensatory mechanisms, such as a decreased conversion of PS to PE or a decreased degradation of PS by phospholipases, might have been induced to maintain a constant level of PS. It is noteworthy, however, that the amount of PSD mRNA was not changed by PSS1 deficiency in brain, heart, or liver. Second, despite the large reduction in total PSS activity, the PSS activity remaining (*i.e.* PSS2) might have been sufficient to maintain a normal rate of PS synthesis.

Because PS appears to play an important role in the brain (for a review, see Ref. 27) we hypothesized that the rate of neuron growth/axon extension might have been reduced by PSS1 deficiency. However, the rate of axon elongation of cultured sympathetic neurons from *Pss1*$^{-/-}$ mice and *Pss1*$^{+/+}$ mice was the same. Similarly we have observed that the rate of axonal elongation of *Pss2*$^{-/-}$ sympathetic neurons is also normal (10). It remains to be determined whether a deficiency of PS or PSS causes subtle disturbances in brain function. These observations demonstrate that PSS2 can substitute for PSS1. Further-

more we have shown previously that the presence of PSS1 in PSS2-deficient mice can, for the most part, substitute for the lack of PSS2 (9, 10). Thus, the observation that *Pss1*$^{-/-}$ mice and *Pss2*$^{-/-}$ mice are viable and outwardly normal implies that there is redundancy in these two synthases and that either isoform is sufficient for mouse viability. Thus, the reason why two distinct genes encoding PS synthase activity exist remains an enigma. It is not clear what evolutionary pressures have preserved the expression of two PS mammalian synthases.

Comparison of PS Synthase-deficient Mice with PS Synthase-deficient CHO Cells—The survival of *Pss1*$^{-/-}$ mice was unexpected in light of previous studies in CHO cell mutants lacking PSS1. Two lines of PSS1-deficient CHO cells have been generated in which the levels of PS and PE were 35–66 and 26–50%, respectively, lower than in wild-type CHO cells (6, 7). Survival of the mutant cells was compromised unless the culture medium was supplemented with either ethanolamine (20 μM) or PS (20 μM). In PSS1-deficient CHO cells, the rate of synthesis of PS and PE, as measured by radiolabeling from [^{14}C]serine, was reduced by ~80%. In addition, the rate of degradation of PS and PE was reduced by ~50% presumably in an attempted compensation for the reduced rate of synthesis of these phospholipids. Moreover the total serine-exchange activity measured *in vitro* was 60% lower in PSS1-deficient CHO cells than in wild-type cells.

Although some of the changes in biochemical parameters of PSS1-deficient CHO cells are similar to those occurring in tissues/cells of *Pss1*$^{-/-}$ mice, there are significant differences that might permit survival of PSS1-deficient mice. First, PSS1 deficiency induced a larger (~80%) decrease in the rate of PS synthesis in the CHO cells than in hepatocytes (20–40%). It is noteworthy that serine-exchange activity measured *in vitro* was reduced to a similar degree in the CHO mutant cells and in PSS1-deficient mouse tissues. Interestingly the CHO mutants, but not the PSS1-deficient hepatocytes, attempted to compensate for decreased production of PS by simultaneously decreasing the rate of PS degradation (6). Second, the decreased PS content of the CHO cell mutants (by 35–66%) was much greater than in PSS1-deficient mouse tissues except for the liver. The PE content of PSS1-deficient CHO cells was also significantly reduced (by 26–50%), whereas the PE content of the mouse tissues was not significantly reduced by PSS1 deficiency. We speculate that the reduced PS and PE content of CHO cells, which was not evident in the mouse tissues, might have been responsible, at least in part, for the decreased survival of the CHO mutants. Third, the mutant CHO cells survived poorly if they were not supplemented with either 20 μM PS or 20 μM ethanolamine; the culture medium of these cells contained smaller amounts of PS, PE, and ethanolamine in the serum that was provided. Although the precise amounts of PS, PE, and ethanolamine that bathe cells of the various mouse tissues are not known, the concentrations of PS, PE, and ethanolamine in mammalian plasma have been reported to be ~100 μM (29), ~100 μM (29), and <2 μM (30), respectively. Thus, we speculate that in PSS1 knock-out mice the amounts of PS and PE circulating in plasma are sufficient to prevent severe adverse consequences of PSS1 deficiency. In contrast, the mutant CHO cells require supplementation of the growth medium with PS or ethanolamine.

Another possible explanation for why PSS1-deficient mice survive and have relatively minor alterations in phospholipid content is that an alternative pathway for PS synthesis, other than the pathways mediated by PSS1 and PSS2, might have been induced in PSS1 knock-out mice. Perhaps this putative pathway was induced to a lesser extent in mutant CHO cells than in the mice. So far, however, there is no evidence that a third PS-synthesizing enzyme exists in mammalian cells.

Mice with Deficiencies in Both PSS1 and PSS2—A *Saccharomyces cerevisiae* mutant (*cho1*) was isolated that lacks PS synthase, has no PS, and grows slowly on rich medium. Growth of this mutant on a non-fermentable carbon source was arrested, and many cells were respiratory-deficient (petite), but the cells remained viable for several days (31–33). Similarly when PS synthase was deleted from *Schizosaccharomyces pombe* the cells lacked PS, grew slowly on rich medium, and exhibited poor viability on minimal medium (34). Thus, PS appears not to be essential for yeast survival. In contrast, our data suggest that mice cannot survive without PS because no *Pss1*$^{-/-}$/*Psss2*$^{-/-}$ double knock-out mice were generated. Nevertheless mice in which three *Pss* alleles were disrupted (*i.e. Pss1*$^{-/-}$/*Pss2*$^{+/-}$ and *Pss1*$^{+/-}$/*Pss2*$^{-/-}$ mice) are viable and appear outwardly normal despite dramatic reductions in total serine-exchange activity of their tissues. Consistent with this decreased capacity for PS synthesis, the PS and PE content of tissues from these mice was significantly lower (by ~40 and ~29%, respectively) than in their wild-type littermates. Clearly mice can function adequately with significantly reduced levels of PS and PE. Presumably, however, there is a minimum threshold level of PS and PE and of PS synthase activity below which viability of mice will be compromised. This lower limit is indicated by studies in the CHO cell mutants with only ~5% of normal total serine-exchange activity (deficient in both PSS1 and PSS2 activity (8). In the absence of PS supplementation the double mutant cells did not survive, and the content of PS and PE was 80 and 49% lower, respectively, than in wild-type CHO cells. Despite only ~5% of normal serine-exchange activity, PS externalization occurs normally on the cell surface of these CHO double mutants during apoptosis (35).

It is not clear, however, whether PS, PE, or both phospholipids are critical for survival. We cannot, at this juncture, distinguish between a requirement for PS *per se* or for PE made from PS via PSD. Our laboratory has reported that the majority of PE in mitochondria is generated *in situ* from PSD rather than being imported from the endoplasmic reticulum where PE is made by the CDP-ethanolamine pathway (5). Similar observations have been made in yeast (33, 36, 37). More recently, our laboratory has demonstrated that mice that are unable to make PE from PSD die during the early stages of development and exhibit severe mitochondrial abnormalities (38), implying that production of at least some PE from PS is required for viability and normal mitochondrial function. Thus, our finding that no PSS1/PSS2 double knock-out mice were born is consistent with the idea that either PS or PS-derived PE is essential for mouse development. Interestingly the production of PE from the CDP-ethanolamine pathway is also required for mouse development

because disruption of the *Pcyt2* gene, which encodes CTP: phosphoethanolamine cytidylyltransferase (39), is embryonically lethal. It is noteworthy that heterozygous mice that contain only a single active allele encoding PSD or CTP: phosphoethanolamine cytidylyltransferase, respectively, are viable (38, 39).

In conclusion, we have generated mice that lack PSS1 and/or PSS2. Elimination of either PSS1 or PSS2 is compatible with mouse development and viability. In contrast, no mice were born that lacked both PS synthases. Our data show that mice can tolerate very low levels of total serine-exchange activity and that this activity can be contributed by either PSS1 or PSS2, indicating significant redundancy in the two pathways of PS synthesis. Furthermore mice can survive with significant reductions of PS and PE content of their tissues.

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Defining the Importance of Phosphatidylserine Synthase-1 (PSS1): Unexpected Viability of PSS1-Deficient Mice

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