Porcine Stress Syndrome: Biochemical and Genetic Basis of this Inherited Syndrome of Skeletal Muscle

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Excitation-Contraction Coupling in Skeletal Muscle

Every time a depolarization of the surface membrane propagates down the transverse tubular network in skeletal muscle, a fast signal transduction process takes place resulting in the release of ionized calcium (Ca^{2+}) from the sarcoplasmic reticulum (Figure 1). This transduction, which occurs in the millisecond to submillisecond time scale, is termed excitation-contraction (EC) coupling (Rios and Pizarro, 1988). Its mechanism is still unknown, but recent advances have led not only to a better definition of the processes involved, but also to the identification of the molecular basis of the inherited skeletal muscle disorder known as Porcine Stress Syndrome (MacLennan and Phillips, 1992).

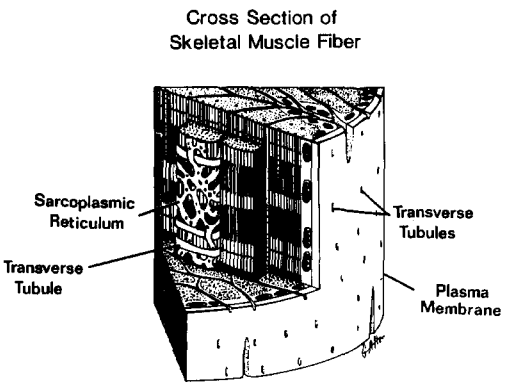
Three processes can be conceptually distinguished in EC coupling: (1) voltage sensing - a process that takes place in the transverse tubule membrane network; (2) transmission, which links voltage sensing to changes in the sarcoplasmic reticulum that allow calcium release; (3) the calcium release process. The specific transverse tubule membrane protein responsible for sensing the voltage in (1) has now been identified to be a calcium channel protein termed the dihydropyridine receptor (Lamb, 1992). This protein is comprised of five different subunits, only one of which contains the voltage sensing and calcium channel activities (Caterall, 1988). In addition, the specific sarcoplasmic reticulum membrane protein responsible for calcium release in (3) has been identified as a completely different type of calcium channel called the ryanodine receptor (Lai et al., 1988). This 560,000 molecular weight protein exists as a homotetramer of two million molecular weight and has been demonstrated to form a large conductance cation channel upon incorporation into planar lipid bilayers (Smith et al., 1988). This receptor complex has been purified in non-ionic detergents, and has been shown to have the morphology identical to the "feet" structures which can be seen to span the transverse tubule-sarcoplasmic reticulum junction (Wagenknecht et al., 1989). A large list of compounds are known to effect sarcoplasmic reticulum calcium release. Potentiators include μM Ca^{2+} adenine nucleotides, caffeine, halothane and nanomolar ryanodine, whereas inhibitors include Mg^{2+}, millimolar Ca^{2+}, calmodulin (Meissner and El-Hashem, 1992) and the only drug that effectively blocks Stress Syndrome response, dantrolene (Ellis and Heffron, 1985). Although there is morphological evidence indicating a close apposition of the ryanodine and dihydropyridine receptors in skeletal muscle (Rios and Pizarro, 1988), the physiological mechanism by which the plasma membrane dihydropyridine voltage sensor (1) communicates to the ryanodine receptor (3) remains to be defined.

A number of animal models have now been identified in which one of the components of EC coupling has been shown to contain a naturally occurring mutation. Such studies have helped us understand the components of EC coupling, and how they function in concert to effect skeletal muscle contraction. The following describes approaches we have taken in our laboratory to understand Porcine Stress Syndrome (Mitchell and Heffron, 1982) that results from a mutation in one of the components of EC coupling.

Porcine Stress Syndrome

Porcine stress syndrome (PSS) and the associated deleterious pale, soft and exudative pork (PSE) carcass traits have plagued the swine industry for many years. Death losses associated with breeding, high temperatures, crowding and transport, plus processing losses due to the PSE meat, are well known (Harrison, 1979). When a stress response is triggered, there is a striking increase in aerobic and anaerobic metabolism, the intense production of heat, CO_2 and lactic

Figure 1



A three-dimensional representation of a skeletal muscle fiber.

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acid and the contracture of skeletal muscles. This syndrome is also triggered following exposure of animals to volatile halogenated anesthetics such as halothane (Hall et al., 1980).

PSS is an inherited syndrome, transmitted by a single autosomal recessive gene. This gene has been variously called the stress gene, *halothane* or *hal* gene, and PSS gene (Hall et al., 1980; Harrison, 1979). The product of the PSS gene was reasoned to be a mutant form of a normal, albeit previously unidentified gene. Two copies (or alleles) of each gene are present in all mammalian genomes. Therefore, with respect to the PSS gene there are three porcine genotypes: homozygous for the normal gene, homozygous for the PSS gene, and heterozygous having a copy each of both the normal and PSS genes.

Biochemical Abnormalities in Porcine Stress Syndrome

Whether or not the resting sarcoplasmic ionized Ca^{2+} concentration is elevated in PSS muscle is still a topic of some debate (Iaizzo et al., 1988). However, a number of different studies have now documented that triggers of PSS result in an elevation of skeletal muscle sarcoplasmic ionized Ca^{2+} concentrations from a resting intracellular Ca^{2+} concentration of approximately 0.2μM. to > 1μM (Iaizzo et al., 1988; Lopez et al., 1992). If the sarcoplasmic Ca^{2+} concentration is not lowered by one of the membranous systems of muscle, this Ca^{2+} increase is sufficient to activate muscle contractile activity, resulting in a contracture of the muscle. The elevated Ca^{2+} will also activate phosphorylase kinase, the key Ca^{2+}-dependent enzyme of glycogenolysis, resulting in an increased glycogen breakdown. The prolonged contracture requires energy which is derived from both aerobic and anaerobic metabolism. Thus, much of the glucose phosphate liberated from muscle glycogen is metabolized anaerobically to lactate resulting in lacticacidosis. The defect in PSS muscle therefore is reasoned to be the result of an altered calcium transport and/or permeability of one of the cell membrane systems of muscle.

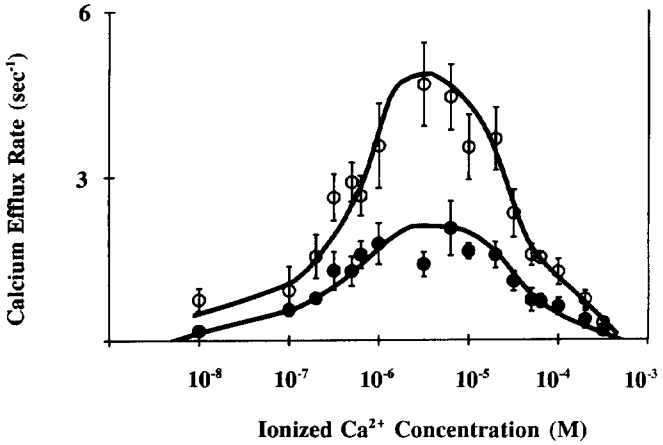
We have examined the calcium transport and permeability processes of the muscle membranes that control sarcoplasrnic Ca^{2+} in both normal and PSS pigs. ATP-dependent calcium uptake and passive calcium permeabilities of muscle surface membranes (sarcolemma and transverse tubules) are unaltered in PSS (Mickelson and Louis, 1985; Ervasti et al., 1989) so calcium entry from outside the muscle cell is unlikely to be responsible for the elevated Ca^{2+} following triggering of PSS. Calcium sequestration by PSS and normal sarcoplasmic reticulum ATP-dependent Ca^{2+} pump are also the same (Mickelson et al., 1986), indicating muscle relaxation is not altered in PSS muscle.

The only functional abnormality that we have identified in PSS muscle membranes is in the sarcoplasmic reticulum calcium release channel protein (Gallant et al., 1989; Knudson et al., 1990; Mickelson et al., 1988; Mickelson et al., 1989; Mickelson et al., 1992; Shomer et al., 1993). Initial studies using isolated porcine muscle sarcoplasmic reticulum vesicles loaded with ^{45}Ca demonstrated that the rate constant for calcium release follows a bell-shaped curve with respect to the ionized Ca^{2+} (Fill et al, 1990) (Figure 2). This calcium-induced calcium release from isolated sarcoplasrnic reticulum membranes of PSS pigs is approximately twice as fast as calcium release from normal sarcoplasmic reticulum membranes (Figure 2). Such an abnormality could explain the elevated Ca^{2+} in PSS muscle if the sarcoplasmic reticulum ATP-dependent calcium pump is unable to match this increased rate of calcium release.

Another approach that has been used to examine the sarcoplasmic reticulum Ca^{2+} release channel function in PSS is to measure the binding of the plant alkaloid ryanodine. The binding of ryanodine has given this calcium channel its more popular name of "ryanodine receptor." This drug only binds to the open state of the calcium release channel so binding provides a measure of the relative activity of the channel (Meissner and El-Hashem, 1992). Ryanodine binding to normal and PSS sarcoplasmic reticulum is significantly different, such that the affinity of ryanodine for its receptor is approximately four times greater in PSS than normal sarcoplasmic reticulum (Ervasti et al., 1991; Mickelson et al., 1988) (Figure 3). Very similar results have also been obtained with the purified receptor protein indicating that the defect responsible for the abnormal ryanodine to the calcium release channel is due to an alteration in this protein and not a membrane-associated lipid or protein (Shomer et al., 1993). The increased affinity of ryanodine for the PSS sarcoplasmic reticulum calcium release channel could be explained by the hypothesis that once opened, the PSS channel appears to be harder to close. Interestingly, the calcium release channel in heterozygous pigs has properties that are intermediate between those of the homozygous stress-susceptible and homozygous normal animals. This we demonstrated with both sarcoplasmic reticulum ryanodine binding and calcium release (Mickelson et al., 1989).

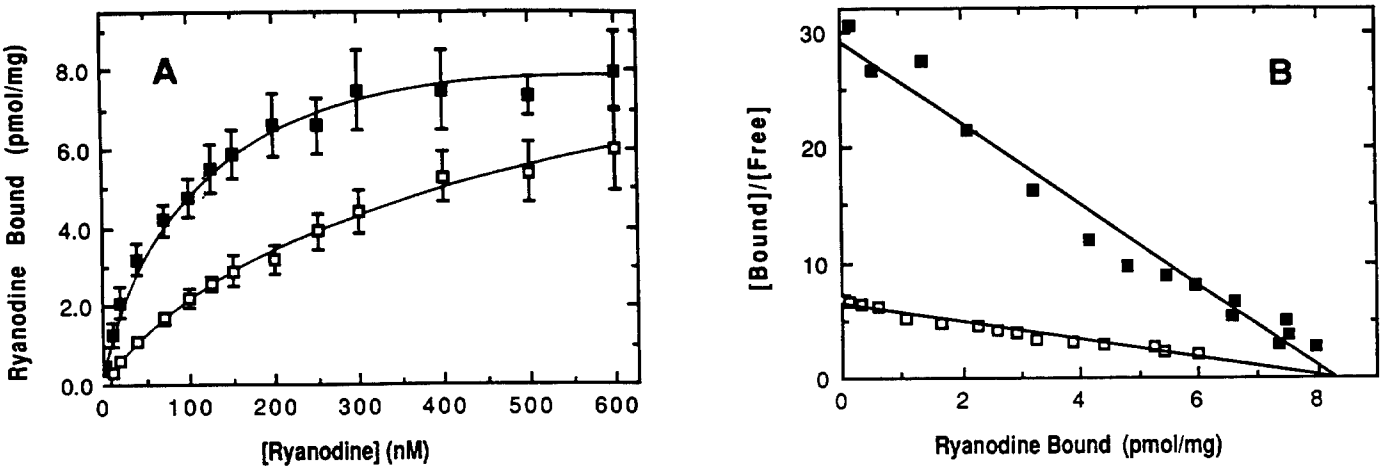
If the sarcoplasmic reticulum calcium release channel is the site of the defect in PSS, then it should be the site of action of PSS triggering agents. We have recently examined this by investigating the effect of volatile anesthetics on calcium release from isolated PSS and normal sarcoplasmic reticulum vesicles (Louis et al., 1992). These studies demon-

Figure 2



Ca^{2+}-dependence of calcium release from PSS (solid circles) and normal (open circles) sarcoplasrnic reticulum vesicles. Points represent ± SE for three preparations each of PSS and normal sarcoplasmic reticulum vesicles. Reprinted with permission of Fill et al. (1990).

Figure 3

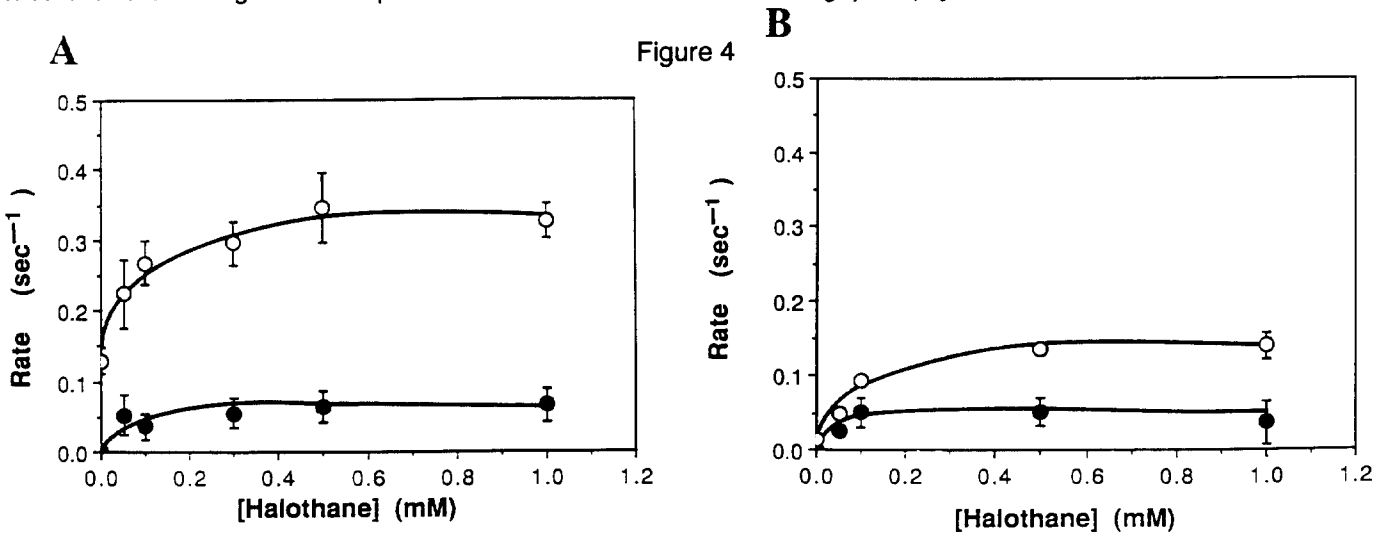


A, specific [^{3}H]ryanodine binding by PSS (filled squares) and normal (open squares) sarcoplasmic reticulum vesicles. *B*, Scatchard plots of [^{3}H]ryanodine binding by MHS and normal sarcoplasmic reticulum vesicles. Points represent the means ±SE of duplicates for each of three different PSS and three different normal sarcoplasmic reticulum preparations. The B_{max}, (maximum ryanodine binding) for normal (8.6 ± 2.2 pmoles/mg vesicles) and PSS (8.5 ±1.2 pmoles/mg vesicles) were not significantly different; the K_d (affinity for ryanodine) for normal (284 ±102 nM) and PSS (62 ±15 nM) were significantly different. Reprinted with permission of Ervasti et al. (1991).

strated that concentrations of halothane that trigger a stress response stimulated sarcoplasmic reticulum calcium release, and that this occurred at sarcoplasmic Ca^{2+} concentration at which these agents trigger PSS in vivo (i.e. at 10^{-7} M Ca^{2+} when muscle is in the relaxed state) (Figure 4). Finally, whereas calcium release from normal sarcoplasmic reticulum is inhibited as pH is decreased below the physiological level (approyamately 6.9), calcium release from PSS sarcoplasmic reticulum still occurred at pH 6.6. This inability of low pH to inhibit calcium release from PSS sarcoplasrnic reticulum may be significant in vivo, where the insensitivity of the PSS sarcoplasmic reticulum to this regulation allows calcium release to continue even during the PSS response. These results also indicate that halogenated anesthetics induce PSS by acting directly on the sarcoplasmic reticulum calcium release channel protein.

These previous approaches, while identifying alterations in the PSS sarcoplasrnic reticulum calcium release mechanism, do not unambiguously examine the activity of this calcium channel protein. However, by analyzing the single channel activity of this protein incorporated into planar lipid bilayers, a number of kinetic characteristics of the channel protein can be characterized, including the percentage of time the channel is in the open or closed states, its selectivity for different types of ions (e.g. monovalent versus divalent cations, or Ca^{2+} versus Mg^{2+}), the physical size of the ion conducting channel,

Figure 4

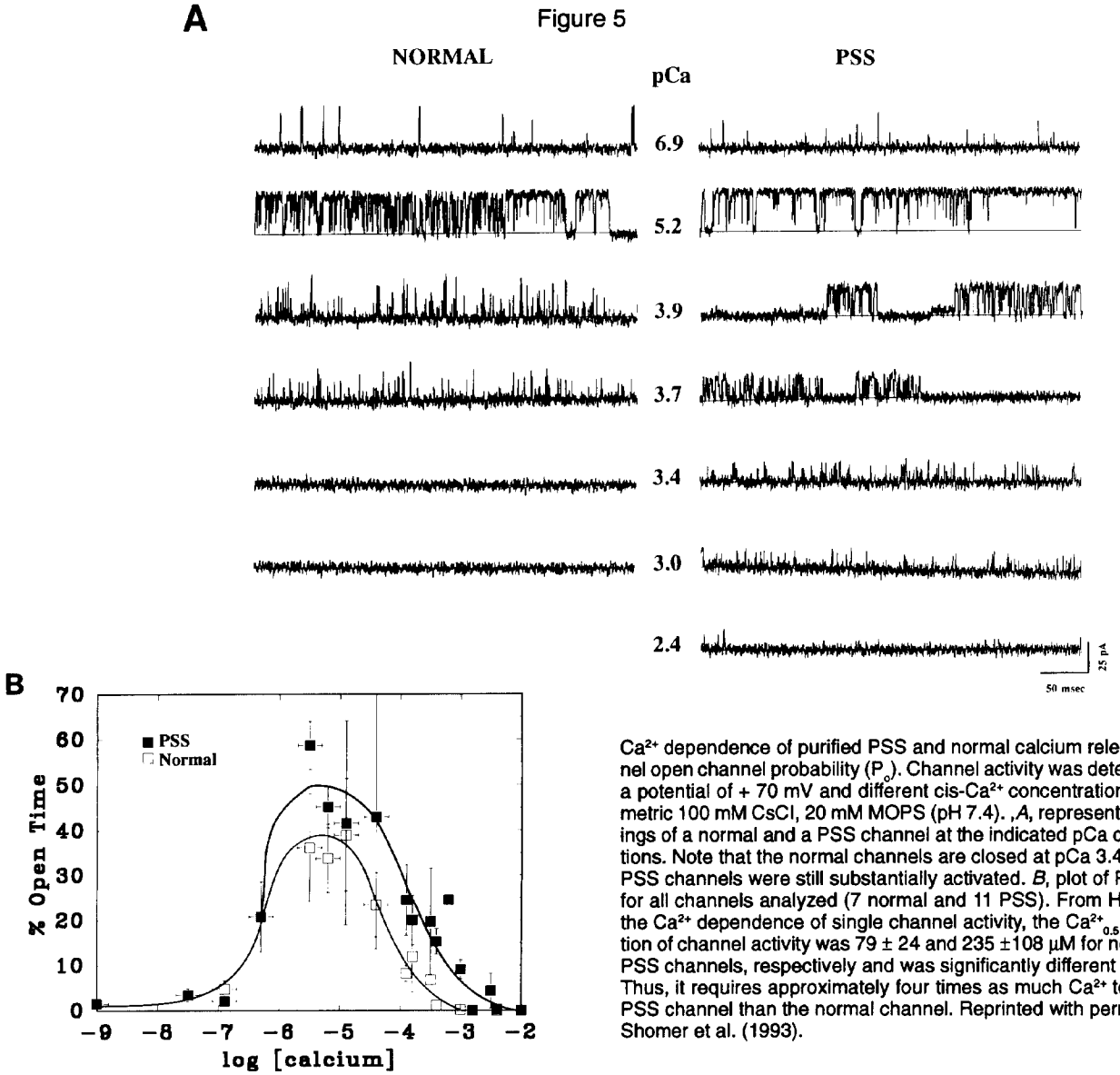


Effect of halothane on the rate constant for calcium release from PSS (*A*) and normal (*B*) sarcoplasmic reticulum. Calcium release was determined by dilution of ^{45}Ca- ' loaded sarcoplasmic reticulum vesicles into various concentrations of halothane in 10^{-6} M Ca^{2+} medium (open circles), or a calcium release inhibiting medium (filled circles). Points represent the means ±SE of duplicate determinations on three PSS and three normal sarcoplasmic reticulum preparations. Note that calcium release is stimulated by clinical concentrations of halothane at resting muscle calcium concentrations (10^{-6} M). Most importantly, the stimulation of calcium release from PSS sarcoplasmic reticulum (*A*) is much greater than from normal sarcoplasmic reticulum (*B*). Reprinted with permission of Louis et al. (1992).

and its dependence on the transmembrane electrochemical gradient. It is in essence the "ultimate" biochemistry experiment as the "gating" (opening and closing) of a single channel molecule can be analyzed. The activity of the PSS and normal channels incorporated into bilayers exhibited a biphasic dependence on Ca^{2+} concentration (Fill et al., 1990; Shomer et al., 1993). The PSS channel was similar to the normal channel in all properties, except for the Ca^{2+} concentration that inactivates the channel activity (Figure 5). Thus, Ca^{2+} concentrations that would close the normal sarcoplasmic reticulum channel in vivo would be unable to close the PSS channel. This then may be the basic biochemical defect responsible for the elevated sarcoplasmic Ca^{2+} concentration in PSS muscle.

It seems reasonable that the different properties of the PSS and normal calcium release channels could be due to structural differences between these two proteins. If so, this could possibly be detected as a difference in the proteolytic sensitivities of the two molecules. Indeed, in a series of studies we have shown that the immunochernically-detected proteolytic fragments of the PSS and normal calcium release channel proteins are different (Knudson et al., 1990; Mickelson et al., 1992). In normal sarcoplasmic reticulum, trypsin generates an 86,000 molecular weight calcium channel peptide that is absent in PSS sarcoplasrnic reticulum (Figure 6). A 99,000 molecular weight calcium channel peptide is generated in both PSS and normal sarcoplasmic reticulum. Proteolysis of heterozygous sarcoplasmic reticulum produces both the 86,000 and 99,000 molecular weight peptides, indicating that both alleles of the ryanodine receptor gene are being expressed in this animal. Furthermore, affinity-purified antibodies to the 86,000 molecular weight polypeptides recognized the 99,000 molecular weight peptides (and vice versa), indicating that both the 86,000 and 99,000 molecular weight peptides are derived from the same region of the calcium release channel protein (Mickelson et al., 1992). Amino acid sequencing of the 86,000 molecular weight calcium release channel peptide of normal sarcoplasrnic reticulum indicated that its N-terminus was residue 616 of the calcium release channel protein. These data could logically be explained by a mutation at amino acid resi-

Figure 5



Ca^{2+} dependence of purified PSS and normal calcium release channel open channel probability (P_o). Channel activity was determined at a potential of + 70 mV and different cis-Ca^{2+} concentrations in symmetric 100 mM CsCl, 20 mM MOPS (pH 7.4). *A*, representative tracings of a normal and a PSS channel at the indicated pCa concentrations. Note that the normal channels are closed at pCa 3.4 while the PSS channels were still substantially activated. *B*, plot of P_o vs. pCa for all channels analyzed (7 normal and 11 PSS). From Hill plots of the Ca^{2+} dependence of single channel activity, the $Ca^{2+}_{0.5}$ for inhibition of channel activity was 79 ± 24 and 235 ±108 μM for normal and PSS channels, respectively and was significantly different ($p < 0.02$). Thus, it requires approximately four times as much Ca^{2+} to shut the PSS channel than the normal channel. Reprinted with permission of Shomer et al. (1993).

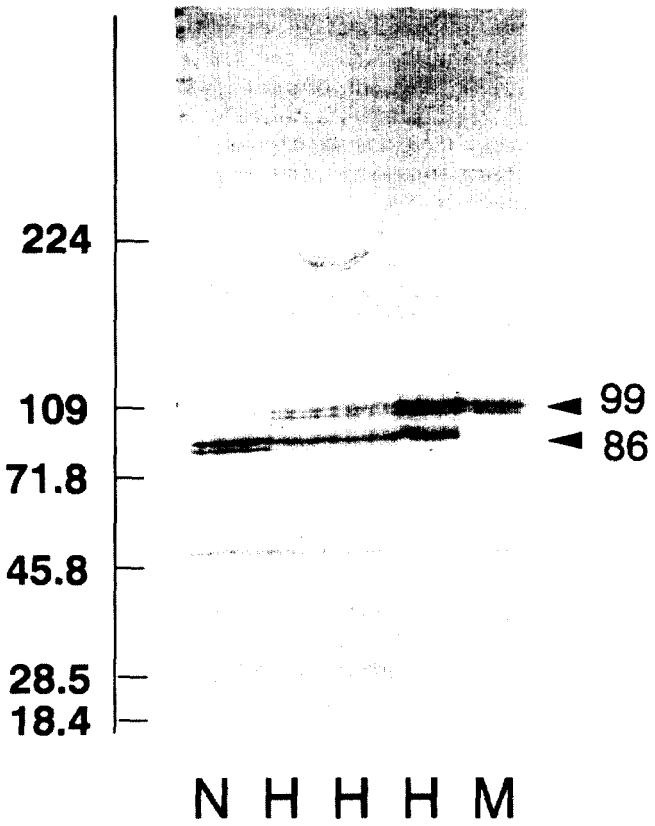
due 615 that eliminated a trypsin cleavage site in the PSS channel protein.

Clinical Diagnosis of PSS

The traditional test for determining PSS has been the halothane-challenge test, whereby susceptible pigs become rigid and undergo metabolic changes in response to administration of this agent (Hall et al., 1980). However, although the homozygous PSS animals can be reliably identified by skilled operators, heterozygotes for the PSS gene are not identified, and continue to carry this gene in the population. In principle, heterozygotes could be identified by backcrossing to a known homozygous PSS animal and halothane-testing the progeny, 50% of which should be halothane positive (homozygous PSS). However, this has not been done on a consistent basis.

MacLennan and co-workers have now conclusively demonstrated that PSS is the result of a mutation in the 5036 amino acid sarcoplasmic reticulum calcium release channel protein (Fujii et al., 1991; Otsu et al., 1991). A single base pair mutation (conversion of a cytosine to a thymine at nucleotide 1843 of the coding sequence) was identified when comparing the DNA sequences of a genetically stress-susceptible Pietrain population with a known non-PSS Yorkshire population (Fujii et al., 1991). This DNA mutation results in the substitution of Arginine to Cysteine at residue 615, and also explains the altered trypsin digestion pattern of the normal and PSS calcium channel proteins (Knudson et al., 1990). The gene for the sarcoplasrnic reticulum calcium channel is now known to be on swine chromosome 6 (segment 6p11-q21) as is the well known *hal* linkage group (Harbitz et al., 1990). Thus, biochemical definition of the abnormality in PSS muscle has converged with classical genetics.

Figure 6



Immunoblot of normal (N), heterozygote (H), and PSS (M) sarcoplasrnic reticulum digested with trypsin. Trypsin-treated sarcoplasmic reticulum was electrophoretically fractionated on polyacrylamide gels, transferred to nitrocellulose membranes and stained with an antibody to the ryanodine receptor protein. The arrowheads on the right show the 86,000 molecular weight fragment which is detected in the normal and heterozygote sarcoplasmic reticulum only, while the 99,000 molecular weight fragment is prominent in the PSS and heterozygote sarcoplasmic reticulum. Reproduced with permission of Knudson et al. (1990).

This discovery of the molecular basis of PSS has in turn facilitated the development of an accurate molecular genetic test for identifying the normal and PSS alleles of the calcium channel protein on the basis of differences in their DNA sequence. The advantage of this molecular test is that we are now able for the first time to determine not only homozygous PSS animals, but also the previously unidentifiable heterozygote animals. This DNA-based test has now been shown to reliably identify the PSS gene in all major breeds of North American pigs (Otsu et al., 1991). Significantly, from detailed haplotyping of the DNA of over 500 animals, MacLennan and colleagues (Fujii et al., 1991; Otsu et al., 1991) have concluded that there was a single founder animal from which PSS originated. The molecular genetic test requires only a sample of DNA which is most readily obtained from the nucleated white blood cells present in a small sample of whole blood. A small fragment of the calcium channel DNA containing the sequences flanking the mutation is amplified using the polymerase chain reaction (Saiki et al., 1985). The resultant amplified DNA sample can then be analyzed to determine whether it contains the nucleotide sequence of the normal gene, the PSS gene, or both (i.e. heterozygote).

There are a variety of different procedures that can be used to identify the mutation in the amplified calcium release channel DNA fragment. However, the most straight forward diagnostic technique takes advantage of the altered specificity of an amplified 733 base pair (bp) DNA fragment to two restriction enzymes. Specifically, the C to T mutation at nucleotide 1843 eliminates a site in the amplified DNA that can be hydrolyzed by Cfo 1, and creates a new site that can be now hydrolyzed by BsiHKA 1. Thus, Cfo 1 only hydrolyses the normal allele of the calcium channel amplified DNA fragment, while BsiHKA 1 hydrolyses at the site of the mutation in the PSS allele. An additional BsiHKA 1 site that is common to both alleles serves as an internal control that provides evidence that the added BsiHKA 1 is active.

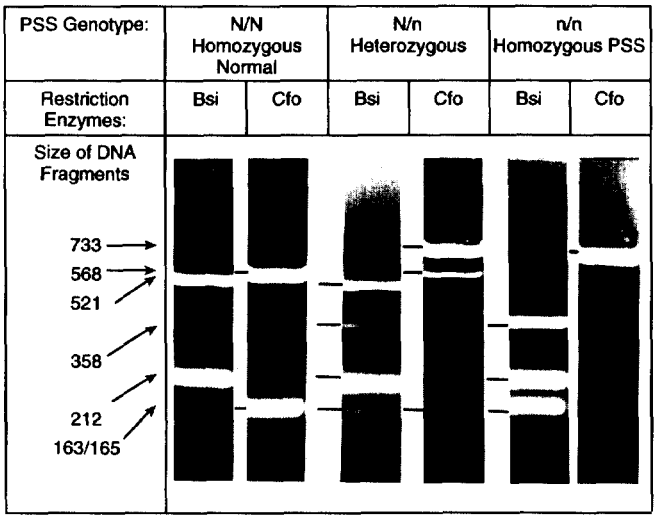
The size of the amplified DNA fragments derived from the three different genotypes, and an example of a typical diagnosis is shown in Figure 7. Restriction enzyme hydrolysis of the DNA amplified from the homozygous normal animal (*N/N*) results in 521 + 212 bp fragments using BsiHKA 1, while treatment with Cfo 1 generates fragments of 568 + 165 bp. Restriction enzyme hydrolysis of DNA amplified from the homozygous PSS animal (*n/n*) results in fragments of 358 + 212 + 163 bp using BsiHKA 1, while treatment with Cfo 1 results in no hydrolysis of the 733 bp amplified product. Restriction enzyme hydrolysis of DNA amplified from the heterozygous ani-

Figure 7

Restriction enzyme fragment size resulting from hydrolysis of amplified DNA

PSS Genotype:	N/N Homozygous Normal	N/n Heterozygous	n/n Homozygous PSS
Restriction Enzymes	Restriction Enzyme Fragment Size (Size in Base Pairs)		
BsiHKA1	521+212	521+358+212+163	358+212+163
Cfo 1	568+165	733+568+165	733

Restriction Enzyme Hydrolysis of the Three PSS Genotypes with BsiHKA1 (Bsi) and Cfo 1 (Cfo)



Restriction Enzyme Hydrolysis of the Three PSS Genotypes with the restriction enzymes BsiHKA I (Bsi) and Cfo 1 (Cfo). The 733 base pair fragment of DNA was amplified from the three different genotypes and then treated with the two different restriction enzymes as indicated. Note that the pattern of digestion by each pair of enzymes is unique for that genotype.

mal (N/n) results in fragments of 521 + 358 + 212 + 163 bp using BsiHKA 1, while treatment with Cfo 1 generates fragments of 733 (the undigested amplified fragment) + 568 + 165 bp. Thus, each PSS genotype has a clearly resolvable, unique pattern of restriction enzyme fragments allowing for the unambiguous permitting diagnosis of the animal's status.

Diagnosis of the Incidence of PSS Gene in Swine Herds

Our laboratory at the University of Minnesota has been using the molecular genetic PSS test and has compared its effectiveness to the traditional halothane challenge test in diagnosing PSS (Rempel et al., 1993). Pietrain swine homozygous for the *hal* gene (*n*) and a Pietrain derivative breed Near Pietrain (NP) with a frequency of 0.31 for *n*, were mated to produce reciprocal F_1, F_2 and purebred NP litters. The halothane challenge test was performed on all 40 parents and 240 progeny to predict their *hal* genotype and PSS susceptibility. The molecular genetic test for PSS was also determined for these animals. The predicted *hal* genotypes observed in the progeny differed significantly from the expected Mendelian ratios, with the halothane challenge test consistently over-

Table 1. Prevalence of the PSS Gene in Samples Provided by U.S. Swine Breeders.

	Number of Animals of Each Genotype		
Producer	*NN*	*Nn*	*nn*
1	24	0	1
2	22	14	3
3	23	7	2
4	20	15	2
5	14	1	0
6	37	4	0
7	21	0	0
8	40	24	0
9	123	44	1
10	28	5	0
11	28	0	0
12	19	0	0

NN is the homozygous normal genotype, Nn is the heterozygous PSS genotype, and nn is the homozygous PSS genotype.

estimating the *n/n hal* genotype. However, the PSS genotypes based on the DNA-based test did not differ significantly from the expected Mendelian ratios, and this DNA-based assay apparently misidentified only one of the 40 parents. The results of the study indicate that the assay for the PSS ryanodine receptor mutation more accurately predicts both the homozygous and heterozygous forms of the PSS gene than does the halothane challenge test.

We have now used this molecular genetic test to monitor the status of prospective breeding animals from some U.S. producers. A sample of the farms in which more than 20 animals were analyzed is indicated in Table 1. Obviously, the incidence of the PSS gene varies from producer to producer, but it is of some concern that it is present, at least in the heterozygote condition, in almost all farms examined to date. This is indicated further in Table 2, which documents that the PSS gene has been detected in US herds of Duroc, Hampshire, Landrace, Large White, Yorkshire, Poland China and Pietrain swine. With the availability of this molecular genetic test for

Table 2. Incidence of PSS Gene in Different Breeds of US Swine.

	Number and Percentage of Animals of Each Genotype by Breed		
Breed	*NN*	*Nn*	*nn*
Duroc	29 (74%)	9 (23%)	1 (2%)
Hampshire	96 (79%)	19 (16%)	7 (5%)
Landrace	210 (64%)	113 (34%)	6 (2%)
Large White	81 (77%)	23 (22%)	1 (1%)
Yorkshire	102 (87%)	13 (11%)	2 (2%)
Pietrain	0	1 (5%)	17 (95%)
Poland China	2	2	0

NN is the homozygous normal genotype, Nn is the heterozygous PSS genotype, and nn is the homozygous PSS genotype.

PSS gene, it will now be important to determine what proportion of PSE meat in commercial operations is directly due to the presence of the PSS gene itself. Indeed, a recent study concluded that the economic contribution of the *hal* gene to the PSE problem was only 4.5%, indicating that preslaughter management practices may be the most important factors contributing to pork quality (Pommier and Houde, 1993). It is known that stresses associated with slaughter as well as variations in processing can lead to poor quality meat even in animals that do not carry the PSS gene (Harrison, 1979). Although it is anticipated that the amount of PSE meat will decline now that the molecular genetic test can be used to selectively remove the PSS gene from all breeding stock, the question remains, how much PSE meat will there still be?

It is also entirely possible that there will also be other effects on the swine industry ff the PSS gene is removed from all breeding stock. It has been known for a number of years that the PSS gene is associated with a range of important production advantages, as it is highly correlated with increased lean meat content and decreased fat content (Harrison, 1979; Mitchell et al., 1982). Thus, previous methods for selection for leaner, highly muscled animals resulted in an increased incidence of PSS. Whether these favorable traits are due to the PSS gene itself, or instead other closely linked genes on swine chromosome 6 is currently being examined (Echard et al., 1992). Given the competitive nature of the U.S. swine industry, it is likely that in the future most breeding stock will be tested to control the PSS gene. While this will allow producers to remove the PSS gene from their stock, it is possible some producers may find it advantageous to create lines for the routine production of slaughter pigs that are heterozygous for the PSS gene, that retain many of the beneficial but not the deleterious traits of the homozygous PSS animals. Alternatively, producers will begin to utilize the many other genes likely to affect muscling in their selection programs. Thus, we are at a very exciting moment for the swine industry, as molecular genetics can now provide producers with a range of previously unavailable options.

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