Mitochondrialer Kalziumausfluß, Schweinestress und Bösartige Hyperthermiesyndrome

K.S. CHEAH

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Arrheniusstellen von kalziumstimulierter Atmung zeigten eine verschiedene Übergangstemperatur in Mitochondrien von stressunempfindlichen und stressempfindlichen Schweinen und deuteten darauf hin, daß die letzteren mehr gesättigte Fettsäuren enthielten. Der Unterschied im anaeroben Kalziumausflußgrad zwischen stress-empfindlichen und -unempfindlichen Schweinen ist wahrscheinlich auf die Erzeugung von Fettsäuren als Folge der kalziumstimulierten Phosphorlipasetätigkeit zurückzuführen. Der wahrscheinliche Mechanismus von Stress und bösartigen Hyperthermiesyndromen wird im Zusammenhang mit den obenerwähnten Ergebnissen besprochen.

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Arrhenius plots of calcium-stimulated respiration showed a different transition temperature in mitochondria from stress-resistant and stress-susceptible pigs, suggesting the latter contained more saturated fatty acids. The difference in the anaerobic calcium efflux rate between stress-susceptible and stress-resistant pigs is likely to be due to production of fatty acids as a consequence of calcium-stimulated phospholipase activity. The probable mechanism for stress and malignant hyperthermia syndromes is discussed in the light of the above evidence.

is not improved by using additional electrodes, positioned for instance in the middle of the back (Savell et al. 1977). However, a practical method of low voltage stimulation could perhaps be developed by stimulating via the spinal cord, e.g. by inserting a plastic pithing rod with electrodes at either end; our preliminary results with this method show that it is less reliable than the standard method, but better, at say 150V (peak) than indicated by the results in Table 2.

Another modification, possibly useful on some slaughter lines, is to stimulate split, dressed sides, preferably within 40 min of slaughter. This method is rather less reliable than stimulation of undressed carcasses (Table 2) but better than stimulation at voltages below 650V.

6. The pathways excited by stimulation

Although very high currents of 4A or more flow during stimulation at 650V.there is no evidence that muscles directly in the current pathway, e.g. the LD and BF muscles, react any more vigorously than the TB muscles in the forelimb which are not in the pathway and in which current flow is minimal. Similarly, when a carcass is hung by one leg and one pair of electrodes is placed on this leg only and the other pair on the neck, vigorous contraction and rapid fall of pH occurs in the free leg in which almost no current flows. This, combined with almost complete absence of contractile response to stimulation of curar sed rabbit and lamb carcasses (Bendall, 1976), shows that very little of the contractile response is due to direct stimulation of the muscles but rather that surviving motor nerve pathways in both the spinal cord and the muscles themselves are activated and are the main cause of the response. In fact, Galvani's (1790) classical experiment of attaching two dead frogs together and stimulating one of them with a bi-metallic strip, whereupon the other frog jumped in time with the first, can be repeated with two beef carcasses touching each other.

Indirect evidence in favour of the almost exclusive implication of nervous pathways in the stimulation process comes from the dying away of the contractile response in rabbit, lamb and beef carcasses within an hour or so of death. At this time the muscle pH is usually high (>6.8) and the ATP-level maximal, so that muscles excised from the carcass can still respond fully to direct stimulation (Bendall, 1977).

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E 2:2

L'écoulement du calcium mitochondrial, le stress porcin et les syndromes d'hyperthermie maligne.

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La mesure de l'écoulement du calcium avec la murexide offre une méthode fiable et sensible pour évaluer la susceptibilité au stress porcin. La mitochondrie du M. longissimus dorsi de diverses espèces de porcs a montré des variations des taux d'écoulement du calcium, les porcs susceptibles au stress montrant un taux d'écoulement deux fois plus élevé que celui des porcs résistants au stress.

Il y a des rapports entre le stress porcin et les syndromes de l'hyperthermie maligne, puisque des taux d'écoulement du calcium élevés s'associent aux porcs sensibles au holothane et aux porcs qui produisent des carcasses post-mortem pâles, molles et suintantes. Il y a une corrélation étroite entre les taux d'écoulement du calcium et le taux de glycolyse, formation de lactate, gouttes, espace extra-cellulaire et hydrolyse de phosphate de créatine et de triphosphate d'adénosine.

Les tracés d'Arrhenius de la respiration stimulée par le calcium ont montré une température de transition différente dans la mitochondrie des porcs résistants au stress et des porcs susceptibles au stress, ce qui donne à penser que ceux-ci contenaient davantage d'acides gras saturés. La difference entre les taux d'écoulement du calcium anéorobique des porcs résistants au stress et des porcs susceptibles au stress provient probablement de la production des acides gras par suite de l'activité phospholipide stimulee par le calcium. Le mécanisme probable des syndromes de stress et d'hyperthermie maligné est discute en tenant compte des constatations.

Выделение кальция из митохондрий, стресс и болезненный гипотермический синдром у свиней

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Метод измерения выделяющегося кальция с помошью мурексида является надежным для исследования чувствительности свиней к стрессовому состоянию. Митохондрии из мускула M.1 longissimus dorsi различных пород свиней показали колебания в темпе выделения кальция: у свиней, чувствительных к стрессу, темп в два раза больше, чем у свиней устойчивых к стрессу. Стресс и гипотермический синдром связаны как с галотеновой чувствительностью свиней так и с побледнением, размягчением и увлажнением туши после убоя. Темп выделения кальция коррелирует с темпом гликолиза, образованием лактата, выделением сока внеклеточного пространства и гидролизом креатинфосфата и аденозинтрифосфата. Графики Аррениуса для окисления, стимулированного кальцием, показали различную переходную температуру в митохондриях у чувствительных и устойчивых к стрессу свиней, что позволяет предполагать большее содержание насыщенных жирных кислот у последних. Разница в темпе анаэробного выделения кальция у чувствительных и устойчивых к стрессу свиней, вероятно, обусловливается образованием жирных кислот как следствие фосфолипазной активности, стимулированной кальцием. Механизм стресса и гипотермический синдром обсуждаются на основании полученных фактов.

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Introduction

The pale, soft and exudative (PSE) condition associated with muscles of stress-susceptible pigs is characterized by rapid biochemical changes immediately after death. The muscles of these pigs show an extremely rapid fall in tissue pH post-mortem, and the ultimate pH value of about 5.5 is attained while the carcass is still hot. This results in denaturation of sarcoplasmic and myofibrillar proteins and consequent exudation of a large amount of drip and pale meat (1-2). Stress-susceptible pigs also show a tendency to develop malignant hyperthermia (3), a condition induced by either halothane (4-8) or by severe physiological stress (9-10). Malignant hyperthermia and PSE appear to be closely related and the mechanism responsible for both these conditions may be identical.

Various researches have been carried out in attempting to explain the cause of PSE condition in stress-susceptible pigs. The early postulation of "uncoupling" of mitochondria (11) and of "aberrant mitochondrial energy metabolism" (12) could not be confirmed by us (13) and by other workers (14-16). Contrary to previous reports (10-11), no marked biochemical difference in the properties of mitochondria from M. longissimus dorsi (LD) of both stress-susceptible and stress-resistant pigs was observed (13-16). Furthermore, no significant difference in either the Ca^{2+}-binding ability of sarcoplasmic reticulum or of myofibrillar ATPase activity were detectable in either stress-susceptible or stress-resistant pigs in LD muscle obtained at 15 minutes post-mortem (17) or from biopsy samples taken with a minimum of muscle trauma (18) to account for the development of PSE meat in stress-susceptible pigs. The recent report of a difference in the sarcoplasmic reticulum between stress-susceptible and stress-resistant pigs (19) could not possibly be due to true difference in the properties of these organelles in situ, but difference derived through acid (pH 5.4-5.7) denaturation of the sarcoplasmic reticulum prior to its isolation from the muscle of stress-susceptible pigs.

In 1976, we suggested that the Ca^{2+} release from mitochondria of LD muscle of stress-susceptible pigs (20) was the 'trigger' for the series of biochemical events responsible for the ultimate formation of PSE and also of malignant hyperthermia syndrome. This suggestion was based on our observation that tightly-coupled mitochondria from LD muscle of stress-susceptible pigs showed an anaerobic Ca^{2+} efflux rate about twice that of stress-resistant pigs, and that halothane only enhanced the mitochondrial Ca^{2+} efflux rate of stress-susceptible pigs. Since that time, we have been investigating the direct relationship between mitochondrial Ca^{2+} efflux and parameters associated with PSE and malignant hyperthermia, and the mechanism responsible for either or both of these porcine syndromes. This paper emphasizes the reliability and sensitivity of the Ca^{2+} efflux measurements in assessing porcine stress-susceptibility, and also describes the probable mechanism for PSE and malignant hyperthermia syndromes.

Materials and Methods

Bovine serum albumin (fatty acid free), rotenone, sodium succinate and murexide (ammonium purpurate) were obtained from Sigma Chemical Corp.; crystalline Bacillus subtilis (Nagarse) from Teikoku Chemical Co., Osaka;

all other reagents were of analytical grade.

All the pigs used were from the stock of healthy animals held at the Meat Research Institute. The halothane-screened Pietrain/Hampshires and Pietrains were kindly supplied by Dr Webb (ABRO, Edinburgh) and Dr Monin (I.N.R.A., Theix) respectively. The weight of the pigs ranged from 70 to 100 kg at slaughter. The mitochondria were isolated from LD muscle using B. subtilis proteinase (20). The Ca^{2+}-stimulated respiration for succinate oxidation was measured polarographically with a Clark oxygen electrode [Yellow Spring Oxygen Monitor (Model 53)] in a total volume of 2.50 ml. The reaction medium (pH 7.20) contained 220 mM mannitol, 50 mM sucrose and 15 mM Tris-HCl in the presence of 5.0 mM P_i. Ca^{2+} uptake was measured by the murexide technique (21) with the Aminco-Chance dual-wavelength/split-beam spectrophotometer operating in the dual-wavelength mode at 540-510 nm at 20°C using the reaction medium described for the oxygen uptake experiments except that 2.50 mM P_i was used. Protein was estimated with Folin-phenol reagent (22) using bovine serum albumin as standard. Tissue pH and metabolites were determined as described by Bendall et al (23).

Results

1. Variations in Stress-susceptibility

In 1976, we showed that different breeds of pigs exhibited different rates of mitochondrial Ca^{2+} efflux (20). This aspect of the work has been extended and substantiated since that period to support the conclusion that mitochondrial Ca^{2+} rates can be used to predict and assess porcine stress-susceptibility. P_i stimulated the rate of mitochondrial Ca^{2+} efflux. Measurements made in the presence of 2.50 mM P_i was found to be very convenient for differentiating porcine stress-susceptibility because of the greater difference observed under these conditions between stress-susceptible and stress-resistant pigs (see Table 1).

Table 1: Rates of Ca^{2+} efflux of LD muscle mitochondria from different breeds of pigs.

The rates of Ca^{2+} efflux refer to the initial fast efflux rate during anaerobiosis (20).
*, Stress-susceptible breed; **, stress-resistant breed; results are means ± SD for the numbers of pigs used in parenthesis. The Ca^{2+} efflux rates between the stress-susceptible and stress-resistant breeds were highly significantly different ($P < 0.001$).

	Ca^{2+} efflux (nmol/min/mg protein at 20°C)	
	- P_i	+ P_i (2.50 mM)
Pietrain*	94 ± 11 (12)	235 ± 29 (12)
Poland China*	83 ± 12 (5)	
Large White**	40 ± 6 (21)	131 ± 12 (10)
Gloucester Old Spot**	32 ± 10 (7)	123 ± 23 (6)

In addition to differentiating stress-susceptibility in different breeds of pigs, Ca^{2+} efflux measurements also show that variation in stress-susceptibility exists within each breed. The data on the Norwegian Landrace pigs (Figure 1) illustrate an excellent example of the occurrence of a wide spectrum in stress-susceptibility in that particular breed. Of the five Norwegian Landrace pigs used, one showed a very fast mitochondrial efflux rate of 220 nmol Ca^{2+} per minute per mg protein (▲), its LD muscle also exhibited the fastest rate of glycolysis (measured by the rate of decline in tissue pH), and the highest amount of drip (3.14%) produced post-mortem.

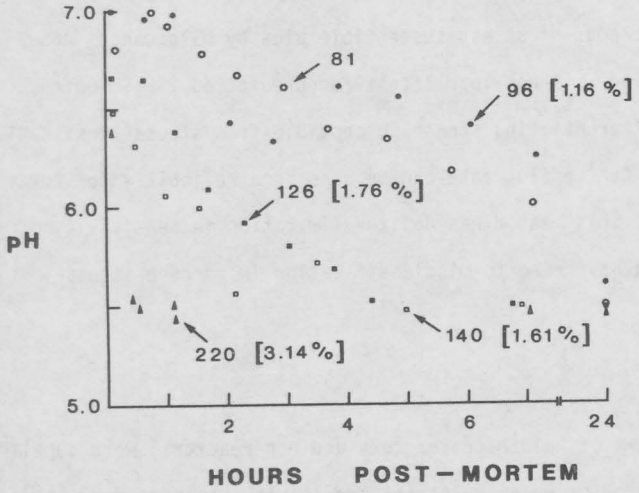

Figure 1 : Relationship between Ca^{2+} efflux, rate of glycolysis and drip in Norwegian Landrace pigs.

Five Norwegian Landrace pigs were used. The values in parenthesis refer to drip at 48 hours post-mortem at $1^{o}C$.

Of the other four pigs, two showed low values in the rate of Ca^{2+} efflux, rate of glycolysis (●,○) and drip (1.16%) the remaining two pigs (■, □) exhibited intermediate values in the three parameters (Ca^{2+} efflux, tissue pH and drip).

Pietrains also showed variation in stress-susceptibility, with some Pietrains showing Ca^{2+} efflux rates similar to those observed for stress-resistant breeds. This observation suggests that not all Pietrains are stress-susceptible (Table 2).

Table 2: Ca^{2+} efflux from LD muscle mitochondria of Pietrain pigs.

The rates were estimated in the absence of P_i; results are means ± SD for the numbers of pigs used in parenthesis.

Breed	Halothane screening	Ca^{2+} efflux (nmol/min/mg protein at $20^{o}C$)
MRI Pietrain	Not screened	94 ± 11 (12)
INRA Pietrain	+	94 ± 15 (5)
MRI Pietrain	Not screened	35 ± 6 (9)
INRA Pietrain	-	42 ± 4 (6)

Nine out of the twenty-one MRI Pietrains exhibited low Ca^{2+} efflux rates which were similar to those of INRA Pietrain halothane non-reactors (values not significantly different between these two groups of Pietrain pigs). Twelve MRI Pietrains showed high Ca^{2+} efflux rates corresponding to rates observed with INRA Pietrain halothane reactors (values not significantly different). The values of the high and low Ca^{2+} efflux rates were highly significantly different ($P < 0.001$).

2. Ca^{2+} Efflux and Halothane Screening

Malignant hyperthermia can be induced in various breeds of stress-susceptible pigs by halothane. Anaesthesia with a mixture of oxygen and halothane was applied as a non-lethal test for predicting PSE in Dutch Landrace pigs (24) and was also widely employed for differentiating stress-susceptible from stress- resistant pigs (25). The following data clearly illustrate that Ca^{2+} efflux rate can be used as a reliable index for predicting porcine stress-susceptibility. Furthermore, this method has not the limitation in sensitivity observed with halothane screening. Halothane screening gave rise to misclassification in porcine stress-susceptibility which could be as high as 15%.

(a) ABRO Pietrain/Hampshires

Full sib pairs of Pietrain/Hampshires (equal numbers of halothane reactors and non-reactors) were supplied by Dr Webb of ABRO. The pigs were killed at MRI, and prediction of halothane sensitivity was made from Ca^{2+} efflux rates prior to knowledge of the results of halothane test carried out by Dr Webb. Three out of twenty Pietrain/Hampshires were misclassified by halothane screening (Table 3).

Table 3: Ca^{2+} efflux from LD muscle mitochondria of ABRO halothane-screened Pietrain/Hampshires. The rates of Ca^{2+} efflux were expressed in nmol Ca^{2+} per minute per mg protein at 20°C in the presence of 2.50 mM P_i. Nine halothane-reactors[(a)] and eight non-reactors[(b)] were correctly classified. The three misclassified pigs are illustrated individually. Ca^{2+} efflux rates for halothane-reactors[(a)] and non-reactors[(b)] were highly significantly different ($P < 0.001$).

Pietrain/Hampshires	Ca^{2+} efflux rate	Prediction from Ca^{2+} efflux	Halothane screening (Webb)
9 pigs	227 ± 24 (9)[a]	+	+
8 pigs	117 ± 17 (8)[b]	-	-
1 pig	252	+	-
1 pig	189	+	-
1 pig	110	-	+

The rates of Ca^{2+} efflux and glycolysis (measured by the decline in tissue pH post-mortem) were directly related in that high Ca^{2+} efflux rates were only associated with muscles showing rapid glycolysis, and low Ca^{2+} efflux rates with muscles having a slow rate of glycolysis (Figure 2). The misclassification by halothane is clearly demonstrated in Figure 3, where a halothane non-reactor (▲) exhibited an almost identical rate of Ca^{2+} efflux and of glycolysis as the halothane reactor (●).

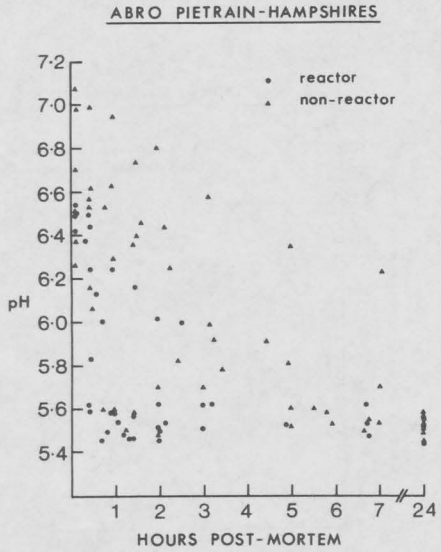

Figure 2 : Relationship between rate of decline in tissue pH post-mortem in LD muscles of halothane-screened Pietrain/Hampshires and Ca^{2+} efflux rates.

● : high Ca^{2+} efflux rates, + reactors

▲ : low Ca^{2+} efflux rates, - reactors.

Data include three pigs misclassified by halothane screening (see Table 3).

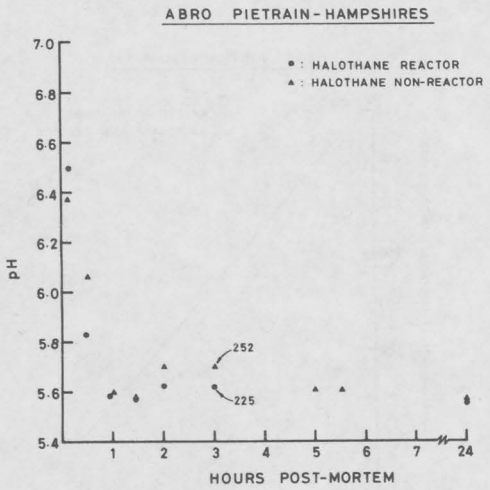

Figure 3 : Relationship between rate of decline in tissue pH post-mortem in LD muscles of Pietrain/Hampshires and Ca^{2+} efflux rates.

The data clearly illustrate the misclassification by halothane screening in that the halothane non-reactor (●) showed similar rate in Ca^{2+} efflux and rate of glycolysis as the halothane reactor (▲).

The Ca^{2+} efflux rates correlated very closely with the amount of lactate formed (Figure 4, correlation coefficient (R), = 0.91), drip produced (Figure 5, correlation coefficient (R) = 0.88),

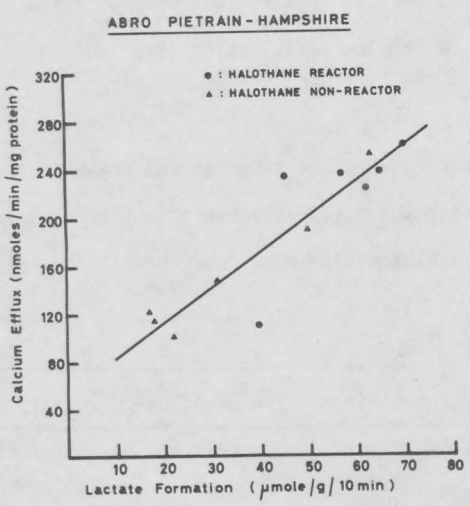

Figure 4 : Relationship between Ca^{2+} efflux and lactate formation.

Twelve pigs were used (six halothane reactors and six non-reactors). Data include two pigs misclassified by halothane screening.

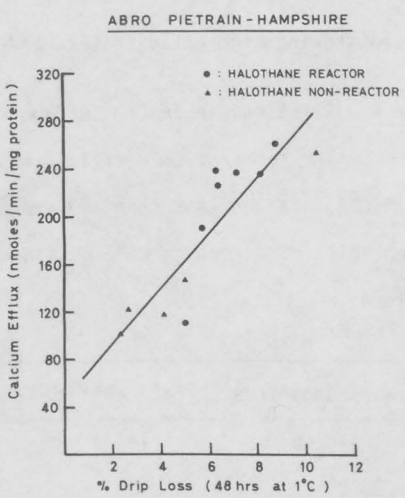

Figure 5 : Relationship between Ca^{2+} efflux and drip produced post-mortem.

The data were obtained using the same pigs described in the legend to Figure 4. Data include two pigs misclassified by halothane screening.

water-binding capacity (Figure 6, correlation coefficient (R) = - 0.80) and extracellular space (Figure 7).

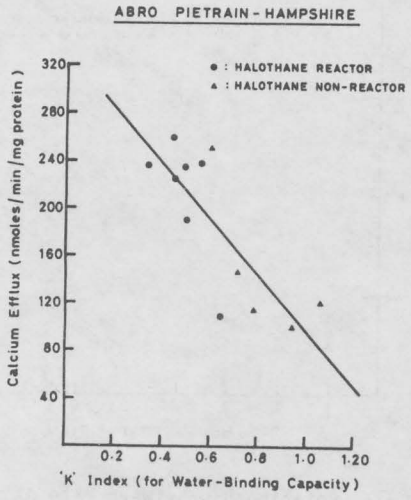

Figure 6 : Relationship between Ca^{2+} efflux and water-binding capacity.

The 'K' index refers to the meat/fluid ratio value obtained by using the press method of Grau and Hamm (26). Values obtained from 24 hours post-mortem sample using the same pigs as described in Figure 4.

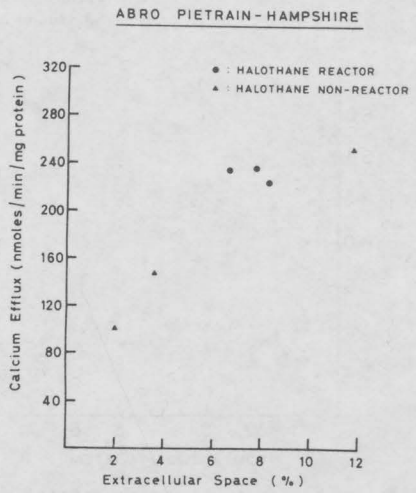

Figure 7 : Relationship between Ca^{2+} efflux and extracellular space.

Six pigs were used (three halothane reactors and three non-reactors). Data were determined from LD muscles at 24 hours post-mortem, and include one pig misclassified by halothane screening.

Ca^{2+} efflux rates were also directly related to the rate of hydrolysis of creatine phosphate and of ATP. High Ca^{2+} efflux rates were only observed in LD muscles showing rapid rates of hydrolysis of creatine phosphate and ATP, and low efflux rates with slow rates of creatine phosphate and ATP hydrolysis.

Ca^{2+} efflux rates correlated very closely with the quality of pork in that PSE was only observed with LD muscles showing high efflux rates, and normal pork with muscles having low efflux rates (see Table 4).

Table 4: Relationship of Ca^{2+} efflux rates and quality of pork

The rates of Ca^{2+} efflux, expressed in nmol/min/mg protein, were estimated in the presence of 2.50 mM P_i at 20°C. Values less than 160 are classified as stress-resistant, and values above this figure as stress-susceptible. The results were obtained from the same ABRO Pietrain/Hampshire pigs described in the legend to Figure 4.

Pietrain/Hampshires	Ca^{2+} efflux	Quality of pork	Pietrain/Hampshires	Ca^{2+} efflux	Quality of pork
1897	147	Normal	1900	237	PSE
2002	101	"	1899	235	"
9914	122	"	2003	225	"
9918	115	"	2005	252	"
9993	110	"	9996	189	"
			9957	239	"
			9890	260	"

(b) ABRO Sire Line Pigs

As in the case of Pietrain/Hampshires, the results of the halothane test on the ABRO Sire Line pigs (27) were unknown to us, but were confirmed after measurements of Ca^{2+} efflux rates with LD muscle mitochondria. One out of the seven halothane-screened Sire Line pigs was misclassified. The LD muscle mitochondria of this misclassified halothane non-reactor had a Ca^{2+} efflux rate of 210 nmol Ca^{2+} per minute per mg protein, which based on Ca^{2+} efflux rate (see Table 1 and Table 3) would be expected to react positively with halothane. The LD muscle of this misclassified pig showed a fast rate of glycolysis, a high value in drip (6.97%) and in extracellular space (13.9%) and was also PSE.

(c) INRA Pietrain Pigs

Ca^{2+} efflux rates of LD muscle mitochondria of six halothane reactors and six non-reactors were measured in collaboration with Dr Monin of Theix. One out of twelve pigs was misclassified by halothane screening in that a halothane reactor showed an identical rate of Ca^{2+} efflux and rate of glycolysis to that observed for halothane non-reactors. With the exception of this single misclassified pig, all the Ca^{2+} efflux rates correlated very closely with the halothane test in that high Ca^{2+} efflux rates were only observed with halothane reactors, which also showed rapid glycolysis post-mortem.

3. Biochemical studies of LD muscle mitochondria

In 1976, we reported the existence of a large difference in the LD muscle mitochondrial Ca^{2+} efflux rates in stress-susceptible and stress-resistant pigs (20). Efforts have been made to seek a logical biochemical explanation for the difference in Ca^{2+} efflux rates and the mechanism responsible for porcine stress and malignant hyperthermia syndromes. Present evidence shows that LD muscle mitochondria from stress-susceptible pigs had a limited capacity to accumulate Ca^{2+} at 40°C, and were also more easily "uncoupled" by Ca^{2+} than mitochondria from stress-resistant pigs. The induced "uncoupling" by Ca^{2+}, which could be prevented by bovine serum albumin, was probably caused by fatty acids released as a consequence of Ca^{2+}-stimulated phospholipase activity at high temperature, and that these fatty acids were also responsible for the enhanced rate of Ca^{2+} efflux at the onset of anaerobiosis in stress-susceptible pigs. Arrhenius plots of Ca^{2+}-stimulated respiration showed that mitochondria of LD muscle from stress-susceptible pigs had a much higher transition temperature than those of LD muscle from stress-resistant pigs (26.6°C ± 0.60 for stress-susceptible pigs (halothane reactors) and 17.6°C ± 0.65 for stress-resistant pigs (halothane non-reactors)). This implied that stress-susceptible pigs probably contained less unsaturated fatty acids in the mitochondrial membranes than mitochondria from stress-resistant pigs (see 28).

Discussion

The reliability and sensitivity of Ca^{2+} efflux as a stress predictor was stringently tested and found to correlate very closely with parameters associated with porcine stress and malignant hyperthermia syndromes. Measurement of Ca^{2+} efflux rates with murexide was shown not to have the limitation of misclassification observed with halothane screening, though the latter method is more convenient and less complicated than that of Ca^{2+} efflux measurements. However, the biochemical procedure for assessing stress-susceptibility is in its

infancy and no doubt could be simplified in the very near future. Ca^{2+} efflux measurements have three distinct advantages over halothane screening i.e. greater accuracy, assessment of a wide range in stress-susceptibility and prediction of PSE condition in some stress-resistant Large White pigs (29; see 25).

The existence of a difference either in the structural or functional integrity of the membranes between stress-susceptible and stress-resistant pigs was shown by the difference in the transition temperature for the Ca^{2+}-stimulated respiration, mitochondrial sensitivity to Ca^{2+} at high temperature, effects of halothane (20), procaine (30) and spermine (31) on Ca^{2+} efflux rates, and bovine serum albumin on Ca^{2+} efflux and Ca^{2+}-stimulated respiration.

The release of Ca^{2+} from mitochondria was postulated by us to be the "trigger" responsible for the ultimate formation of PSE and malignant hyperthermia (20). The evidence just presented further substantiates our original hypothesis. PSE and malignant hyperthermia syndromes can be explained in the following manner. At the onset of anaerobiosis, Ca^{2+} is rapidly released from mitochondria. In stress-susceptible pigs, the transport of Ca^{2+} from the mitochondrial matrix through the inner to the outer mitochondrial membranes activates the phospholipase resulting in the release of fatty acids, which then enhances the initial rate of Ca^{2+} efflux. The sarcoplasmic reticulum is unable to fully accumulate the sudden burst of Ca^{2+} from mitochondria into the sarcoplasm. The excess Ca^{2+} is then free to activate the phosphorylase kinase (32) and myofibrillar ATPase resulting in an overall enhanced rate of glycolysis. The initial failure of the sarcoplasmic reticulum to fully compensate the excess release of mitochondrial Ca^{2+} could be due to the following factors. Firstly, the enhanced rate of Ca^{2+} release from mitochondria of stress-susceptible pigs is twice that of stress-resistant (i.e. normal) pigs. Secondly, inactivation of sarcoplasmic reticulum activity by fatty acids released from mitochondria as a consequence of the Ca^{2+}-activated phospholipase activity. Thirdly, induction of a further release of Ca^{2+} from the sarcoplasmic reticulum by the fatty acids released from mitochondria. Fourthly, further inactivation of the sarcoplasmic reticulum by H^{+} (as a consequence of rapid glycolysis under the prevailing anaerobic conditions), and high temperature (33). Thus the overall effect initially triggered by the mitochondrial Ca^{2+} is ultimately responsible for the rise of serum Ca^{2+} in porcine malignant hyperthermia (34). PSE is brought about by rapid glycolysis in low pH carcasses at high temperature resulting in denaturation of sarcoplasmic and myofibrillar proteins. As in malignant hyperthermia, anxoia-induced Ca^{2+} release from mitochondria is the "trigger" for the series of biochemical events ultimately leading to the formation of PSE.

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