

Mitochondrialer Kalziumausfluß bei halothanbehandelten Schweinen

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Vergleichende Studien über den Kalziumausfluß aus Mitochondrien, die vom M.semitendinosus (weiß) und M.longissimus dorsi halothanempfindlicher und -unempfindlicher Schweine isoliert wurden, wurden mit Murexid durchgeführt. Mitochondrien, die aus beiden Muskeltypen isoliert wurden, wiesen ähnliche Kalziumausflußgrade im anaeroben Zustand auf; bei halothanempfindlichen Schweinen lagen die Grade viel höher als bei halothanunempfindlichen Schweinen. Die Höhe des Kalziumausflusses korrelierte eng mit dem Rückgang des pH-Werts im Gewebe post mortem.

Mitochondrial calcium efflux in halothane-screened pigs

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Comparative studies of calcium efflux from mitochondria isolated from M.semitendinosus (white) and M.longissimus dorsi of halothane sensitive and halothane insensitive pigs were made using murexide. Mitochondria isolated from both types of muscle showed similar calcium efflux rates during anaerobiosis; in halothane sensitive pigs the rates were much higher than in halothane insensitive pigs. The rate of calcium efflux correlated closely with the rate of decline in tissue pH post-mortem.

E 5:2

L'écoulement du calcium mitochondrial dans les porcs traités au halothane.

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Des études comparatives de l'écoulement du calcium de la mitochondrie isolée de M.semitendinosus (blanc) et du M. longissimus dorsi des porcs sensibles au halothane et des porcs insensibles au halothane ont été effectuées en utilisant de la murexide. La mitochondrie isolée des deux sortes de muscles a montré des taux d'écoulement du calcium semblables au cours de l'anaérobiose; dans les porcs sensibles au halothane, les taux ont été beaucoup plus élevés que dans les porcs insensibles au halothane. Le taux d'écoulement du calcium avait une corrélation étroite avec le taux de déclin du tissu pH post-mortem.

Истечение кальция митохондрий у свиней отобранных с помощью галотана

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Сравнительные испытания по истечению кальция из митохондрий выделенных из полусухожильной (белой) и длинейшей спины мышц свиней чувствительных и нечувствительных к галотану проводились с употреблением мурексида. Митохондрии выделенные из мышц обоих типов имели подобные скорости истечения кальция; у свиней чувствительных к галотану скорости истечения были гораздо больше чем у нечувствительных свиней. Скорости истечения кальция тесно совпадались со скоростями падения pH тканей после убоя.

Mitochondrial Ca²⁺ efflux in halothane-screened pigs

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Introduction

The earlier report of Cheah and Cheah (1) suggested that mitochondrial Ca²⁺ efflux could be the 'trigger' for the ultimate formation of pale, soft and exudative (PSE) condition as well as for malignant hyperthermia in stress-susceptible pigs; a large difference in the initial rate of Ca²⁺ efflux from mitochondria isolated from M. longissimus dorsi (LD) of stress-susceptible and stress-resistant pigs was observed. Cheah (2), at this congress, presented evidence on the sensitivity and reliability on measurement of the Ca²⁺ efflux rates from LD muscle mitochondria in predicting stress-susceptible from stress-resistant pigs. He also showed that halothane-sensitive pigs had a much higher mitochondrial Ca²⁺ efflux rate and rate of glycolysis than halothane-insensitive pigs.

This paper shows that mitochondria prepared from the white portion of M. semitendinosus (ST) showed a similar Ca²⁺ efflux rate to mitochondria isolated from LD muscle of the same pig. Thus, measurement of the Ca²⁺ efflux rates from mitochondria of ST (white) could also be used to differentiate porcine stress-susceptibility.

Materials and Methods

Sodium succinate and murexide (ammonium purpurate) were obtained from Sigma Chemical Corp.; crystalline Bacillus subtilis proteinase (Nagarse) from Teikoku Chemical Co., Osaka, and all other reagents were of analytical grade. All the halothane-screened pigs (Pietrain/Hampshires and Sire Line (3)) used were kindly supplied by Dr. A.J. Webb of the Agricultural Research Council, Animal Breeding Research Organisation (ABRO), Edinburgh. The pigs were transferred to the Meat Research Institute, Langford, where they were kept until slaughtered at about 75 kg. They were electrically stunned (90 volts, 50 Hz for 15 seconds), and stuck in the usual manner. ST and LD muscles were excised from the carcass as soon as possible after death (usually within 5 minutes), and the mitochondria were isolated using B. subtilis proteinase (1). The rate of Ca²⁺ efflux from mitochondria was estimated with murexide (4) in an Aminco-Chance dual-wavelength/split-beam spectrophotometer operating in the dual-wavelength mode at 540-510 nm (1). 4-6 mg mitochondrial protein was used in a total volume of 2.70 ml in a 10 mm light-path cuvette; other experimental details were as described previously (1). Protein was determined with Folin-phenol reagent (5) using bovine serum albumin as standard. The pH was measured with a Radiometer pH meter (combined electrode) using muscle homogenate preparations. About 2 g samples of LD muscle were taken from the carcass at various time intervals with a cork borer (15 mm diameter) and the pH determined in 5 mM iodoacetate-150 mM KCl buffer (pH 7.0).

Results and Discussion

Table 1 summarizes the comparative data on the rate of Ca²⁺ efflux from mitochondria isolated from ST (white) and LD muscles of halothane-sensitive and halothane insensitive pigs. The data clearly illustrate that

E 5:4

mitochondria from ST (white) and LD muscles have similar Ca^{2+} efflux rates, and that high Ca^{2+} efflux rates in either type of muscle occurred only in halothane-sensitive pigs. As these experiments were carried out prior to knowledge of the classification of the halothane-screening test, Ca^{2+} efflux rate would have been successful if used as a predictor of stress-susceptibility.

Table 1 : Rate of Ca^{2+} efflux from ST (white) and LD muscle mitochondria of halothane-screened pigs

The rates of Ca^{2+} efflux were estimated in the presence of 2.50 mM P_i at room temperature (20°C), and values less than 160 nmol/min/mg protein are classified as stress-resistant (see Cheah (2)). +, stress-susceptible; -, stress-resistant; paired t-test not significant.

ABRO Pigs	Ca^{2+} efflux (nmol/min/mg protein)		Prediction for stress-susceptibility by	
	ST (white)	LD	Ca^{2+} efflux measurement	Halothane screening
Sire Line	184	191	+	+
Sire Line	221	189	+	+
Pietrain/Hampshire	203	239	+	+
Pietrain/Hampshire	213	234	+	+
Sire Line	123	87	-	-
Sire Line	127	147	-	-
Pietrain/Hampshire	114	115	-	-

Cheah (2), and Cheah and Cheah (6) had observed that mitochondria with a high Ca^{2+} efflux rates were associated only with muscle showing a fast rate of glycolysis (measured by the decline in tissue pH post-mortem) and other parameters (such as drip, water-binding capacity) linked with PSE condition. These observations are supported by the rates of glycolysis measured in LD muscle in halothane-sensitive and halothane-insensitive pigs (Figure 1).

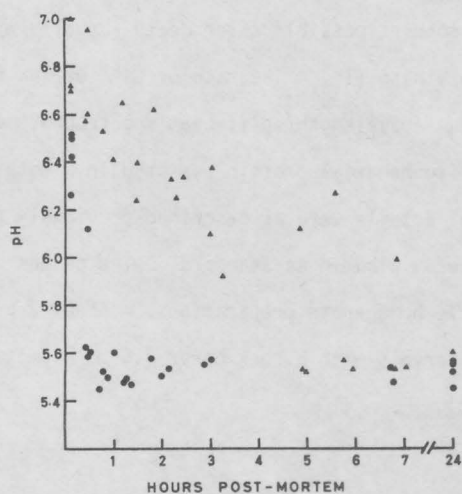


Figure 1 : The rate of decline of pH post-mortem in LD muscle of pigs with high and low Ca^{2+} efflux rates

- (4 pigs) : high Ca^{2+} efflux, + ve reactors
- ▲ (3 pigs) : low Ca^{2+} efflux, - ve reactors.

Measurement of the initial rate of Ca^{2+} efflux of mitochondria from ST (white) during anaerobiosis with murexide thus provides an alternative muscle to LD (Cheah and Cheah (1), Cheah (2), Cheah and Cheah (6)) for assessing porcine stress-susceptibility. The reliability and sensitivity using the Ca^{2+} efflux method devised by Cheah and Cheah (1,6) against the halothane screening is further substantiated by Cheah (2) at this congress.

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