DIES ON 2,3-DIPHOSPHOGLYCERATE, PYRUVATE KINASE AND MITOCHONDRIAL ATPase ACTIVITIES IN PIG

G TSAO CHEN, CHIH PING LEE and DENG CHENG LIU
rtment of Animal Science, National Chung-Hsing University
ung, Taiwan 40227, Republic of China

MARY: The experiment was conducted to study the metabolism of 2,3-DPG and pyruvate kinase (PK) and mitochondrial ATPase activities PSS pig. LYD three crossbred pigs were screened into normal and PSS pigs with Halothane test, which were used as test animals. The samples obtained from the stressed animals for determining DPG and PK activity before slaughtered, and Longissimus dorsi muscle was for measuring mitochondrial ATPase activity 30 min postmortem.

the blood in the PSS pig was lower than that of the normal pig (6.70 $\pm$ 0.25 and 7.16 $\pm$ 0.19, respectively). The concentration of DPG in of the PSS pig was lower than that of the normal pig. This result was in agreement with the structure of DPG changed or dissociated in the of the PSS pig. PK activity in the PSS pig was higher than in the normal animals. However, no difference in mitochondrial ATPase was ed in the stressed and the normal pigs. The result was also found there was difference in DPG concentration between male and female pigs the groups, but no difference in PK activity was detected in different sex.

RODUCTION:

Since DPG is very important factor for maintaining normal physilogical function of oxygen transportation. It can bind globin and lower the affinity of hemoglobin and oxygen under normal condition. As blood flow through the muscle HbO_2 release O_2 to e for aerobic metabolism. However, when the chemical structure and concentration of DPG change, it may retard HbO_2 release O_2 to de muscle needs. Therefore the muscle will turn aerobic metabolism into anaerobic metabolism, and produce lactate which will lower pH of e and blood (Chiba and Sasaki, 1978). The phenomenon is similar to the findings of PSS pigs when they are suffered stress. Meanwhile, of glycolysis takes place very fast in PSS pigs. Whether this is caused by Pyruvate Kinase action, since it is a very important enzyme for olling rate of glycolysis. So its activity should be studied. And the ATP depletes very fast in PSS porcine muscle thus ATPase in ondria is also measured (Yang et al., 1991). In the past years, research workers all stressed on work of catecholamine, corticosteroid halothane test and CPK assay (Cassens et al., 1975). Since no differences were found in the concentrations of catecholamine, costeroid between normal and PSS pigs. Thus, the purpose of this study is to use ^{31}p-NMR and spectrophotometry and biochemical surements to study the 2, 3-diphosphoglycerate, pyruvate kinase and mitochondrial ATPase activities of PSS pigs, as compared to the normal

TERIALS AND METHODS:

issimus dorsi muscle and blood specimen were obtained from the normal and PSS pigs which were slaughtered at local slaughterhouse. The d specimen added with anticoagulant-heparine and EDTA for measuring DPG and PK. The muscle samples were placed in liquid nitrogen for ondrial ATPase activity measurement. DPG content was determined according to the method described by de Verdier and Ericson (1981), K activity was measured with the procedures of Fujii and Miwa (1984).

P-spectra were measured by NMR-spectrophotometer (7.05 Tesla, Varian Instrument Ltd, USA). Specimen of venous blood was collected heparinized tubes and mixed with D_2O and keep the tubes at room temperature for NMR-spectra measurement. Mitochondria isolation and ondrial ATPase activity were carried out according to the procedures described by Cain and Skilleter (1987).

SULTS AND DISCUSSION:

of blood from PSS pigs was lower than that of the mormal pigs. Blood proteins has buffering ability so it pH does not drop below 5.8 like the de of the PSS pigs. The pH of the normal pig was 7.16 and the PSS pigs was 6.70 in the blood (Table 1).

rd specimen for NMR-spectra analysis was collected from the PSS and normal pigs which were suffered stress with electrical shock. From the MR spectra of blood, it could be observed the structure of 2,3-DPG was changed in the blood of PSS pigs after stress, but not observed in blood of the normal pigs (Fig. 1). DPG which was shown in Fig. 1 indicated the structure of PSS pigs stressed with electrical shock seemed e dissociated or might be converted into glycerol-3-phosphate.

2,3-diphosphoglycerate (DPG) content in the blood of the PSS pigs was averaged at 3.885 mmole per liter of blood (male 3.40, female n mole/l) which was lower than the blood of the normal pigs with 5.925mmole/l (male 5.71, female 6.15 mmole/l) (Table 1). The result ated that the DPG content in female pig was higher than that of the male pigs both the normal and PSS pigs. This result was in accordance the structure or NMR-spectra of DPG changed or dissociated in the blood of the PSS pigs shown in the Fig. 1.

levels of the glycolytic enzymes were regulated mainly by three enzymes---hexokinase, phosphofructokinase, and ptruvate kinase which were key enzymes responsible for the regulation of glycolysis. Therefore, one of thses three major enzymes, pyruvate kinase activity in blood of pigs was measured in this experiment. Pyruvate kinase activity in the blood of the PSS pigs was higher than that of the normal pigs. They

were 41.9 unit/ml red cell for male and 45.1 unit/ml red cell for female; and the normal pigs were 20.4 unit/ml red cell for male and 19.4 unit/ml red cell for female (Table 1).

Pyruvate kinase activity can catalyze the conversion of PEP to pyruvate with regen-eration of ATP. Since ATP is also important for the maintenance of cell integrity, it is not surprising that highly sophisticated mechanisms for regulating levels of adenine nucleotides have evolved. As was stated above, the importance of ATP to energy metabolism of red cell, whether ATPase activity was related to physiological properties of muscle from PSS pig and normal pigs. Therefore, mitochondrial ATPase activity in muscle for the normal and PSS pigs was measured. The mitochondrial ATPase activity in normal pork was higher than that of PSE pork. They were 0.1575umole pi/min/mg protein and 0.1509 u mole pi/min/mg protein for the normal pork and PSE pork, respectively. No difference was detected between the normal and PSE pork. In conclussion, pH, DPG content of blood of PSS pig were lower than the normal pig, but PK activity of the blood in PSS pig was higher than that of the blood in the normal pig, however, no difference in mitochondrial ATPase in muscle was detected between the normal and PSS pigs.

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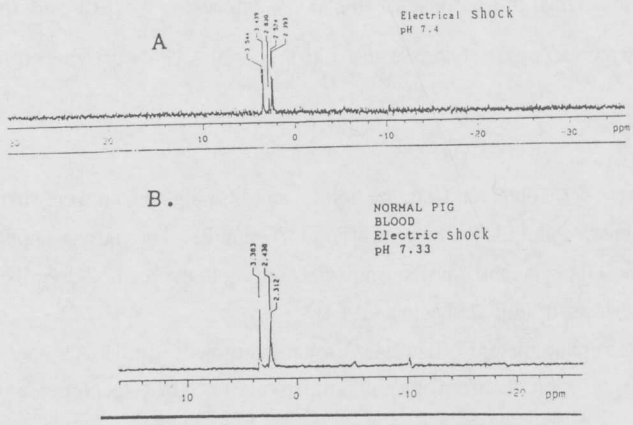

Fig 1 ^{31}P-NMR spectra of blood from PSS and the normal pig (A)PSS (B)normal.

Table1 .Some selected Biochemical characterics of blood and mitochondrial ATPase in muscle of the normal and PSS pig.

	PH	ATPase umole pi/min/mg prot.		DPG mmole/l blood	PK unit/ml RBC
Normal	7.16±0.19[a]	0.1575±0.0080	♂	5.71±0.20[ab]	20.4±1.3[a]
			♀	6.15±0.09[a]	19.8±1.8[a]
PSS	6.70±0.25	0.1510±0.0105	♂	3.40±0.14[b]	41.9±3.3
			♀	4.07±0.19	45.1±3.8

Value given are the mean±the standard error

[a]there are significantly different between two groups($p<0.01$)
[b]there are significantly different between male and female($p<0.01$)