ROLE OF MITOCHONDRIAL CALCIUM IN THE PORCINE STRESS SYNDROME

by

D. G. Topel

The porcine stress syndrome (PSS) is characterized by several abnormal signs which develop when pigs are subjected to noxious stimuli. Stress from extreme physical exercise or extreme temperatures and humidities can trigger the syndrome. Several clinical signs are usually observed when pigs develop the porcine stress syndrome. Muscle tremors are an early sign of stress adaptation difficulty and can be considered one of the first clinical signs. Further stress can result in marked dyspnoea, irregular breathing, rapid increase in body temperature, cyanosis and development of an extreme lactic acidosis condition. The last stage of the syndrome results in a total collapse, marked muscle rigidity, hyperthermia and death occurs while the pig is in a shock-like state (Topel et al., 1968).

In 1966, Hall et al., reported that certain pigs can develop a malignant hyperthermia syndrome (MHS) when exposed to certain anesthetics. The clinical features are (1) gross muscular rigidity, (2) rapid rise in body temperature, (3) trachycardia, (4) hyperventilation and (5) blotchy cyanosis. In addition, there is a severe metabolic (lactic acid) acidosis and a rapid rise in some serum electrolytes, particularly calcium and potassium (Berman, 1970; Harrison, 1973). The malignant hyperthermia syndrome is triggered by certain inhalation anesthetics such as halothane and depolarizing skeletal muscle relaxants (Moulds and Denborough, 1974).

Research at the Iowa and Illinois Experiment Stations (Rasmussen and Christian, 1976) would indicate that the porcine stress syndrome and the malignant hyperthermia syndrome in pigs is the same genetic abnormality. Several studies have indicated that skeletal muscle is the primary defective tissue for triggering the syndromes because of the rapid depletion of skeletal muscle ATP, rapid production of lactic acid and development of extreme muscle rigidity and heat (Topel et al., 1968; Denborough et al., 1970; Kalow et al., 1970 and Nelson et al., 1972). The true lesion(s) of this condition must control, therefore, the above signs. In the last four to five years, there has been a growing amount of research relating Ca^{2+} with the skeletal muscle abnormalities which trigger the development of the porcine stress syndrome (Campion and Topel, 1975). In this paper, I will review some of the research on mitochondria function and the control of Ca^{2+} by mitochondria in skeletal muscle cells. I will relate these topics to the malignant hyperthermia and porcine stress syndromes.

A brief review of mitochondria structure and function may be helpful before I explain the role of Ca^{2+} in the PSS. Mitochondria have two membrane systems, an outer membrane and an extensive, highly folding inner membrane.

Important functions of the mitochondria are (1) to bring about the aerobic oxidation of the products of hydrolysis of lipids and proteins, and of the glycolysis of carbohydrate, coupled with the synthesis of ATP from ADP and Pi, and (2) to provide reducing equivalents for synthetic reactions that take place in the cytoplasm outside the mitochondria with the help of ATP which is largely made by the mitochondria. For example, 32 of the 36 ATP's formed when glucose is completely oxidized are generated through the mitochondria (Slater, 1971).

Oxidative phosphorylation is the process in which ATP is formed as electrons are transferred from NADH or FADH_{2} to O_{2} by a series of electron carriers. Oxidative phosphorylation is carried out by respiratory assemblies that are located in the inner membrane of mitochondria. The citric acid cycle and the pathway of fatty acid oxidation take place in the adjacent mitochondrial matrix. These two processes supply most of the NADH and FADH_{2} in skeletal muscle cells (Racker, 1968).

The oxidative phosphorylation mechanism also influences the regulatory controls of muscle glycolysis. Studies by Eikelenboom and van der Bergh, 1973 and Hellrion et al. (1977) indicated a lower respiratory control of the mitochondria from skeletal muscle of pigs which can develop the PSS and MHS signs. This would result in a lowered potential for the formation

*D. G. TOPEL

Department of Animal Science, Iowa State University, Ames, Iowa 50011.
of energy rich compounds (ATP, creatine phosphate) and an indirect stimulation of muscle glycolysis. Eikelenboom and van der Bergh (1973) suggested that the lowered respiratory control of the mitochondria could be the major abnormality in the skeletal muscle of stress-susceptible pigs.

Further studies by Cheah (1973), Brooks and Cassens (1973) and Campion et al. (1975, 1976) do not support the hypothesis suggested by Eikelenboom and van der Bergh. Data from these studies indicate small and non-significant differences for the respiratory control index in skeletat muscle mitochondria from normal and stress-susceptible pigs. These small differences could not possibly account for the very large differences in ATP depletion (Wang et al., 1969; Berman et al., 1970; and Berman and Kench, 1973) and lactic acid production (Berman et al., 1970 and Weiss et al., 1974) during the middle and final stages of the stress syndrome.

Recently, another function of muscle mitochondria has been associated with the abnormalities which could trigger the stress syndrome. The abnormality is associated with the rate of Ca$^{2+}$ binding by the mitochondria (Heffron et al., 1977) and the rate of Ca$^{2+}$ efflux (Cheah and Cheah, 1976).

Research reported by Vasington and Murphy (1962) and DeLuca and Engstrom (1961) clearly illustrated that mitochondria can accumulate large, net amounts of Ca$^{2+}$ from the suspending medium during electron transport. Moreover, it was found that Ca$^{2+}$ stimulates respiration of mitochondria in a stoichiometric and cyclic fashion, in such a manner that 2 Ca$^{2+}$ ions yield the same amount of extra oxygen uptake as 1 molecule of ADP (Chance, 1963, 1965; Rossi and Lehninger, 1964).

At least two sets of respiration-independent Ca$^{2+}$-binding sites exist (Reynafarje and Lehninger, 1969). One set has a rather low affinity for Ca$^{2+}$ but is very numerous. This type of Ca$^{2+}$ binding is believed to involve non-specific anionic binding groups of membrane proteins and lipids. The other set, however, is much less numerous and has a very high affinity for Ca$^{2+}$ (Chance and Azzi, 1969).

Because of its Ca$^{2+}$ accumulating ability, mitochondria may play a significant role in the excitation and relaxation of muscle, either supplementing the established role of the sarcoplasmic reticulum in segregation of Ca$^{2+}$ or possibly even supplementing it in some types of muscles. In particular, the high affinity and specificity of the Ca$^{2+}$ transport mechanisms in mitochondria may enable them to function in the release and segregation of Ca$^{2+}$ in red muscles, which are profuse in mitochondria but have a rather sparse sarcoplasmic reticulum, whereas the sarcoplasmic reticulum may be dominant in this function in white muscles, which have fewer mitochondria but are rich in reticulum (Patriarca and Carafoh, 1969; Haugaard et al., 1969; and Lehninger, 1970).

The rate of Ca$^{2+}$ release from skeletal muscle mitochondria may play an important role in triggering or at least contribute to the triggering mechanisms for the development of the porcine stress syndrome signs. Cheah and Cheah (1976) reported that halothane enhanced the rate of Ca$^{2+}$ efflux by at least twice and the rate was much higher in the stress-susceptible Pietrain pigs than the more stress-resistant Large Whites. This could contribute to an excess of Ca$^{2+}$ in the sarcoplasm. The excess Ca$^{2+}$ is free to activate the myofibrillar ATPase (Campion et al., 1976) and it could also activate the phosphorylase kinase (Ozawa et al., 1967) so that glycogen is degraded to pyruvate. As the stress syndrome reaches the middle and final phases, the skeletal muscle cells become more anaerobic and more of the pyruvate derived from glycogen is converted to lactate.

Also, Cheah and Cheah (1976) suggested that the enhanced glycolysis triggered by excess Ca$^{2+}$ can result in an increase in phosphoenolpyruvate which in turn could induce Ca$^{2+}$ efflux from mitochondria of stress-susceptible pigs. The induction of Ca$^{2+}$ efflux by phosphoenolpyruvate was reported by Chudaonjge and Haugaard (1973) and McCoy and Doeg (1975).

The role of the sarcoplasmic reticulum must also be mentioned when Ca$^{2+}$ regulation of skeletal muscle is discussed because the majority of the Ca$^{2+}$ accumulating capacity of skeletal muscle is in the sarcoplasmic reticulum (Sulakhe et al., 1973). Nelson (1977) proposed a mechanism which would involve the sarcoplasmic reticulum as one of the triggering agents for the malignant hyperthermia syndrome. Nelson's theory is based on the actions of the muscle relaxant dantrolene. Dantrolene appears to have its main effect by blocking the mechanism coupling depolarization of the sarcolemma membrane to calcium release from the sarcoplasmic reticulum (Putney and Bianchi, 1974). This mechanism is commonly referred to as the excitation-contraction coupling mechanism. Nelson (1977) suggested that the excitation-contraction coupling mechanism can be regulated by at least two sites. The signal produced at Site A is transmitted through Site B. The signal released at Site B causes calcium release from the sarcoplasmic reticulum for production of twitch tension response. The amount of signal produced by Site B is propor-
tioned to that released from Site A. Halothane is agonistic and dantrolene is antagonistic (Nelson and Denborough, 1977) for Site A. The effect of dantrolene in blocking the halothane potentiation of muscle twitch was an indication that both agents act on the excitation-contraction coupling mechanism.

Harrison (1975) and Gronert et al. (1976) have demonstrated the value of dantrolene in preventing the development of malignant hyperthermia in susceptible pigs. Dantrolene apparently blocks the abnormal excess signal from Site A in sensitive pigs when they are given the anesthetic halothane. This reduces the release of Ca\(^{2+}\) from the sarcoplasmic reticulum and reduces the degree of contracture and rigidity of skeletal muscle, reduces stimulation for greater ATP depletion and lactic acid production by controlling the amount of Ca\(^{2+}\) in the sarcoplasm.

The two proposed theories (lesions in the control of mitochondrial or sarcoplasmic reticulum Ca\(^{2+}\)) are interesting and appear to be important in attempting to explain the true lesion which can trigger the stress syndrome. The theories are based on limited research and a need exists to develop further studies to explain more fully the genetic abnormality which can trigger the stress syndrome. It appears that the lack of proper control of membraneous portion of skeletal muscle is important in triggering the stress syndrome but the specific defect is still a mystery.

REFERENCES


