PREVENTION OF STRESS AND HALOTHANE-INDUCED MUSCLE DAMAGE IN PIGS.

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Introduction. Malignant hyperthermia is associated with a myopathy especially in stress-susceptible pigs, but also in man and other animal species. It is triggered by drugs and environmental stress (high temperature, emotional excitement, exercise and muscle injury). Triggering drugs include potent inhalation anaesthetics, skeletal muscle relaxants and local amide anaesthetics. Triggering results in muscle stiffness (especially in pigs), hyperthermia, tachycardia, hyperventilation, mottled cyanosis of the skin and metabolic acidosis. The clinical syndrome is aggravated by sympathomimetics, parasympatholytics, cardiac glycosides and calcium salts. Amelioration is obtainable by calcium lowering agents. The abnormality that could account for the rapid rise in myoplasmic calcium concentration is not yet known; the possibilities are multiple. A defective accumulation of calcium in the SR (sarcoplasmic reticulum) or the mitochondria, and/or an excessively fragile sarcolemma with passive diffusion of calcium into the myoplasm from the extracellular fluid are possible mechanisms of action.

We showed that 5-HT_2 antagonists can prevent halothane-induced malignant hyperthermia and also exercise-induced muscle damage in stress-susceptible pigs.

Method. 1. Halothane-induced malignant hyperthermia. Piétrain pigs (+ 20 kg body weight) were injected with 5-HT_2 antagonists or isotonic saline before (-30 min) exposure to 12 min halothane anaesthesia (4% halothane during first 5 min followed by 2-3% halothane). Clinical observations (cyanosis and muscle stiffness) were combined with measurement of rectal temperature. Blood samples and muscle biopsies were taken before and 19 min after start of anaesthesia. In a few pigs the systolic, diastolic blood pressure and the venous distensibility were also measured by plethysmography. The central venous pressure, the respiratory rate and the tidal volume were continuously recorded.

2. Exercise induced muscle damage. Belgian Landrace pigs and Piétrain pigs (+ 80 kg) were injected with 5-HT_2 antagonists or with isotonic saline 20 min before running on a treadmill for 10 min (speed: 6 km/h). Each pig was tested with saline and with 5-HT_2 antagonists. Blood samples (venous blood) were taken before running, immediately after running and 4 hours later from a catheter (jugular vein) implanted at least 10 days before the first exposure to exercise stress.

Results. Halothane-induced malignant hyperthermia susceptible pigs pretreated with 5-HT_2 receptor blocking agents (0.25-0.5 mg/kg) all survived a subsequent exposure to halothane. No muscle stiffness was observed. Rectal temperature increased by 0.2 to 0.4°C or decreased below the initial rectal temperature. Measured plasma values did not change significantly. Without pretreatment, all pigs showed the typical clinical syndrome of MH: muscle stiffness 30 sec to 3 min after the application of halothane, hyperthermia (42.5-44°C) and blotchy cyanosis of the skin. All pigs died after a variable time period (12-40 min). Plasma values of potassium, calcium, inorganic phosphate, lactate and cholesterol were significantly increased. Electron microscopy of muscles of non pretreated pigs showed swollen mitochondria with a clear increased calcium content. In pretreated pigs no morphological damage was seen. The calcium content of the mitochondria was only moderately increased. In control pigs, exposed to halothane, a significant decrease in venous distensibility accompanied with a high increase in peripheral resistance and central venous blood pressure was seen. This increase in peripheral resistance was accompanied with a significant increase of arterial blood pressure (systolic and diastolic). A few minutes before the animals died, arterial blood pressure (systolic and diastolic pressure) decreased sharply. A decreased cooling of the muscles by a decreased blood flow through the muscles and the skin is most probably involved in the development of hyperthermia. Pigs pretreated with a 5-HT_2 antagonist activity showed an increase of the venous distensibility and no significant increase of the peripheral resistance. Arterial and central venous blood pressure did not change significantly. In the treadmill experiments, pigs pretreated with isotonic saline showed after 10 min a significant increase of blood lactate, base excess, potassium and inorganic phosphate. After 4 hours, LDH and CPK values were significantly increased. These blood values had only moderately increased after pretreatment with 5-HT_2 antagonists. The % of increase depends on the potency of the substance as a 5-HT_2 antagonist and on other pharmacological properties of the same substance (alpha 1 antagonism, dopamine antagonism, alpha 2 agonist activity).

Discussion. 5-HT_2 antagonists prevent the development of malignant hyperthermia in susceptible pigs and also muscle damage in pigs submitted to physical stress. In vitro our substances did not show a protective activity on halothane (1%) nor on caffeine (2 mM) induced contractions. The response was stated on isolated muscles in vitro. In vivo, stress and the sympathetic nervous system are obviously intimately involved in MH and muscle damage in pigs. Piétrain pigs are extremely sensitive to alpha adrenergic stimulation and develop a fatal hyperthermia. Serotonin has shown to be involved in the stimulation of motorneurones and also in the development of tremor and myoclonus. Serotonin has been shown to reverse blockade induced by the nondepolarising agents and hemicholinium and to potentiate blockade produced by the depolarisers. All of these neuromuscular actions of serotonin can be prevented by the administration of methysergide. In rats combination of imipramine (5-HT uptake blocker) plus serotonin resulted in a pattern of muscle necrosis resembling strongly the lesions of Duchenne-type muscular dystrophy. Serotonin is also involved in muscle necrosis in the functional ischaemic skeletal muscle and also in the development of degenerative changes in the skeletal muscle. What is most important in the development of MH: a primarily abnormal membrane structure as suggested by in vitro experiments or an increased release or decreased metabolism of serotonin or both? In experimental animals it has been shown that halothane inhibits the uptake of serotonin in the lungs and by the platelets. Also a direct involvement of serotonin on the labilization of skeletal muscle membrane is possible. Serotonin is also involved in the increase of peripheral resistance and decrease of local blood flow. The local hypoxia, as a consequence of decreased peripheral blood flow, certainly plays a role in the damage of cell membranes and necrosis of the muscle cells as shown by electron microscopy and also in the anaerobic metabolism as shown by the high lactate content. More details will be presented.

Conclusion. With our substances, we can prevent the whole syndrome of MH and exercise-induced muscle damage.

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