Title: EFFECTS OF CAFFEINE ON TRANSMEMBRANE POTENTIAL IN HALOTHANE-PRETREATED HEART PREPARATIONS ISOLATED FROM MALIGNANT HYPERTHERMIA SUSCEPTIBLE PIGS

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Introduction. During human and porcine malignant hyperthermia (MH), cardiac arrhythmias and altered myocardial function can be observed.[1] It is still unknown whether or not a primary abnormality in the cardiac muscle contributes to the cardiac symptoms during MH. Recently, evidence for a direct myocardial involvement in MH was reported for the pig model.[2] This in-vitro study demonstrated abnormal halothane effects on excitation-contraction coupling in the ventricle of MH susceptible (MHS) swine based on weak alterations on the transmembrane potential. The purpose of the present study was to investigate the capability of caffeine to enhance such abnormal effects.

Methods. MHS Pietrain and non-MHS Yorkshire (control) swine (each group n = 6) were used as experimental animals. Swine were halothane challenge-tested for MH susceptibility 4 weeks prior to the experiments. The anaesthetized animals (metomidate, 10 mg/kg ip) were killed by a blow on the head and by bleeding from the carotid arteries. Right ventricular trabeculae were dissected from the freshly excised hearts in oxygenated tyrode solution and the preparations (diameter <1mm, length 3-10 mm) were mounted in a recording chamber (volume 0.8 ml), which was continuously perfused with tyrode solution (composition in mmol/l): NaCl 140, KCl 4.7, $CaCl_2$ 3.2, $MgCl_2$ 1.0, glucose 6.0, $NaHCO_3$ 11.4, NaH_2PO_4 0.38) and continuously gassed with 97% O_2 +3% CO_2. Temperature was maintained at 35°C, the pH was 7.4 and the flow rate through the chamber was 8 ml/min. The muscles were driven at 1 Hz by rectangular voltage pulses 4 ms in duration and about 10% above threshold voltage through one concentric bipolar platinum electrode close to the base of the muscle. Glass microelectrodes filled with 3 mol KCl were used to impale muscle fibers and to monitor the transmembrane potential. Analog differentiation of the action potential (AP) was obtained with an electronic differentiator. AP and upstroke velocity were monitored simultaneously on an oscilloscope (Tektronix, D 13) and converted to digital form (Tektronix, 5 D 10 waveform digitizer) for the final evaluation. After a drug-free equilibration period of 60 min the muscles were pretreated with halothane (1%) over 30 min. Halothane was introduced into the reservoir of tyrode solution by passing the oxygen-carbon dioxide mixture through a calibrated vaporizer. The halothane concentration was kept constant while caffeine was applied cumulatively to the organ bath at 5 min intervals. Data are presented as mean values ± S.E.M. Statistical analysis was performed by paired or unpaired t-test. Differences were considered significant for $p<0.05$.

Results. The electrophysiologic parameters are summarized in the table. AP measurements in the preparations from both MHS and control pigs resulted in small differences when subjected to halothane alone. Additional application of caffeine in cumulative doses (1, 2 and 4 mmol/l) yielded specific dose-dependent alterations of the AP shape for each group: In non-MHS preparations, AP was slightly depressed in amplitude (APA) accompanied by an increase of AP duration (APD) at the 50 and 90% repolarisation level. In the muscles of MHS pigs however, AP was increased in amplitude and markedly widened as expressed by a significantly more pronounced prolongation of APD_{50} and APD_{90} as well as an increase of APD_{20}. In both muscles, caffeine induced a slight decrease of the upstroke phase ($\dot{V}_{max}$) and resting membrane potential (RMP).

Discussion. The present data indicate that caffeine augments abnormal halothane effects on the cardiac transmembrane potential of MHS swine and demonstrate clear differences between the AP configuration in the MHS and the control group. The application of caffeine, in the presence of halothane, results in a slight reduction of AP amplitude and an increase of AP duration in the isolated myocardium of normal pigs in contrast to a plateau enhancement and a marked prolongation of the phase III repolarisation in the heart muscle of MHS pigs. These intensified abnormal responses to a combination of two agents used to test the MH susceptibility of skeletal muscle further support the theory of a myocardial abnormality in MHS pigs.

References.

1. Gronert GA, Theye RA, Milde JH, Tinker JH. Catecholamine stimulation of myocardial oxygen consumption in porcine malignant hyperthermia. Anesthesiology 49:330, 1978

2. Roewer N, Rumberger E, Kochs E, Schulte am Esch. Effects of halothane on excitation-contraction coupling in isolated heart muscle of malignant hyperthermia susceptible swine. Anesthesiology 65:A242, 1986

Table

	caffeine conc.	APA (mV)	RMP (mV)	APD_{90} (ms)	APD_{50} (ms)	APD_{20} (ms)	$\dot{V}_{max}$ (V/s)
non-MHS	control	126±3	91±3	194±7	160±6	69±15	174±18
	1 mmol	125±4	91±3	209±13*	172±7*	77±8	172±17
	2 mmol	122±3*	91±3	225±12*	183±10*	81±10	170±19
	4 mmol	120±3*	88±4*	236±12*	192±15*	81±11	166±19*
	Δ%	-4.2±1.3	-3.5±3.1	21.6±4.6	19.9±9.0	28.8±30.3	4.5±1.6
MHS	control	137±4	92±2	226±14	186±9	87±17	189±1
	1 mmol	139±4°	91±2	248±21*°	206±17*°	101±13°	182±17
	2 mmol	142±4*°	91±2	282±32*°	234±28*°	111±9°	180±14*
	4 mmol	143±4*°	89±1*	303±26*°	253±29*°	119±15*°	175±14*
	Δ%	3.6±1.2	-2.3±1.8	33.5±10.3	35.5±10.3	39.8±25.5	-7.2±3.0

each group n = 6, Mean ± SD, * $p<0.05$ compared to control values, ° $p<0.05$ MHS vs. non-MHS group with caffeine application

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