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Variation in the expression of different heat shock proteins mRNA in EDTA stabilized blood of pigs exposed to different stunning methods (#653)

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Introduction

Heat shock proteins (HSPs) are present in all organisms and in all cells of all organisms [1]. They are essential molecular chaperones in protein assembly [2] and disassembly, protein folding and unfolding [3], and refolding of damaged proteins [4-7]. Diverse stressors induce the expression of HSP genes in muscle cells [8-11]. Hsps belong to multigene families that range in molecular size from 10 to 150 kDa and are found in all major cellular compartements [8]. The Hsp family can be divided in five major groups based on their size, structure and function: (1) Hsp110; (2) Hsp90; (3) Hsp70; (4) Hsp60 and (5) small Hsp (sHSP) families [12].

In sticking blood different short term stressors could affect mRNA levels of hsps, which starts with the actual transport conditions up to stunning [13]. In case of the electrical stunning the pigs have to be isolated and were fixed in a stunning box. At the CO_2 stunning the initiating phase is the major stressor for the pigs. CO_2 anesthetizes excellently but its mucosal averseness is a problem in the introduction.

Therefore the aim of the present study is to evalute the mRNA expression levels of different heat shock proteins in pigs blood exposed to different stunning methods.

Methods

1. Animals, experimental design and sampling

On ten different days, 140 slaughter pigs (guilts and barrows) with an live weight of 118 ± 22 kg were randomly selected at the slaughterhouse Kulmbach. 73 pigs were stunned electrically using operator-handled tongs (head stunning: 1.5 A, 5 s; head-heart stunning 1.3 A, 6 s). 67slaughter pigs were stunned with CO_2 using a Dip-Lift system (95 % CO_2, 100 s) (Butina, Denmark). The blood samples were collected as mixed blood from arteries and veins during exsanguination. Blood samples were collected in blood tubes (10 mL Sarstedt, Germany) containing EDTA (1.6 mg/mL blood), and immediately chilled on ice. The EDTA stabilized blood samples were frozen and stored at -80°C.

2. Isolation of the total RNA and synthesis of cDNA

Total RNA was isolated from 400 µl EDTA stabilized blood using 1200 µl Lysis Blood buffer (Roboklon, Germany) according to manufacture's instructions. 5 µl of each sample was synthesized in to cDNA using NG dART RT KIT (Roboklon, Germany) following the manufacture's instructions, and then stored at 4 °C until use.

3. Quantitative PCR (qPCR)

All experiments were performed on a Biorad qPCR Cycler CFX 96 (Biorad, Germany). Each DNA sample (10 µl) was suspended in 2x SG qPCR Master Mix (Roboklon, Germany) with primer (10 pmol/ µl of downstream and upstream primer, respectively) and RNA free water to a total volume of 25µl. The primers were synthesized by Metabion (Germany) (Table 1). The themal profil was established according to the manufacturer's protocol. Briefly, this protocol was 95 °C for 10 min for enzyme activation, followed by denaturing the doubled stranded DNA at 95 °C for 15 s, annealing at 59 °C for 30 s and elongation at 72 °C for 30 s, for a total of 41 cycles. Finally a inactivation for step was included at 95°C for 15s. For each run, a negative control without DNA was running along with the experimantel samples.

Results

The aim of the study was to evalute the mRNA expression levels of different heat shock proteins in pigs blood exposed to different stunning methods. Therefore all data sets were performed in duplicates. The means of these duplicates were normalized to house keeping genes (GAPDH and ß-Actin). All of the 140 slaughtered pigs, which represent common German crossbred lines, could be analyzed and evaluated. The examined groups did not pass the test on normal distribution; therefore, the Mann-Whitney test was carried out for the statistical evaluation. This test was highly significant ($P<0.001$) for the respective comparisons despite the large individual animal differences. The delta ct-values demonstrated a huge animal individually range, which are shown as box plot diagrams in Figure 1. A gender-specific evaluation did not take place. The highest delta ct-value (17.9383) was detected for hsp70-CO_2 and the lowest (-1.9380) for hsp70- E. It is noticeable that the median of the delta ct-values for hsp27 for electrical stunning are above the value of CO_2 stunning, whereas it is the opposite for the other tested hsps.

Conclusion

Heat shock proteins can be detected in the blood of slaughtered pigs; however, they show very large animal-individual differences. To be able to use hsps as stress markers is either the definition of reference ranges for resting values or the correlation with other better studied parameters such as adrenaline or noradrenaline essential.

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Notes

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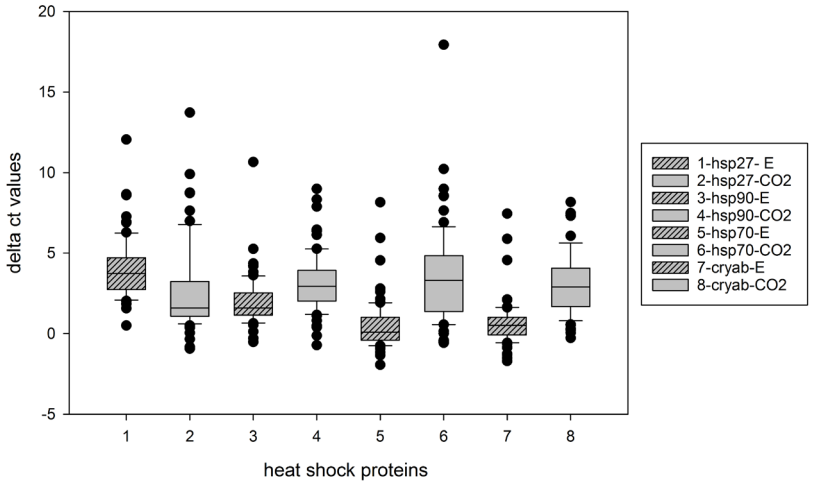

Figure 1: Box plot diagrams of the delta ct values of the different examined heat shock proteins and

	name	sequence 5'-3´	basepair length	NCBI reference number
GAPDH	GAPDH-for	CAGCAATGCCTCCTGTACCA	70 bp	NM_001206359
	GAPDH-rev	GATGCCGAAGTTGTCATGGA		
ß-Actin	ß-Actin-for	CCCTGGACTTCGAGCAGGAG	102 bp	DQ452569.1
	ß-Actin-rev	GCTCGTTGCCGATGGTGATG		
hsp27	hsp27-for	CCTGGACGTCAACCACTTC	123 bp	AY789513.1
	hsp27-rev	GTGAAACACCGGGAAATGA		
hsp70	hsp70-for	CTCAGACCTCTTCCGCAGCA	200 bp	NM_001123127
	hsp70-rev	CACCATAGGCCACAGCCTCA		
hsp90	hsp90-for	TGGAGTTCAGAGCCCTTCTT	108 bp	U94395.1
	hsp90-rev	TGATGAACACTCTGCGAACA		
alphaB	cryab-for	TCTTTGGAGAGCACCTGTTG	122 bp	EU518771.1
crystallin	cryab-rev	CTCTGAGAGCCCAGTGTCAA		

Table 1: Overview of the primers (sequence, length of the amplified DNA frag-ment and NCBI reference

Notes