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Genome-wide analysis of the heat stress response in Zebu (Sahiwal) cattle

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ABSTRACT

Environmental-induced hyperthermia compromises animal production with drastic economic consequences to global animal agriculture and jeopardizes animal welfare. Heat stress is a major stressor that occurs as a result of an imbalance between heat production within the body and its dissipation and it affects animals at cellular, molecular and ecological levels. The molecular mechanism underlying the physiology of heat stress in the cattle remains undefined. The present study sought to evaluate mRNA expression profiles in the cattle blood in response to heat stress. In this study we report the genes that were differentially expressed in response to heat stress using global scale genome expression technology (Microarray). Four Sahiwal heifers were exposed to 42 °C with 90% humidity for 4 h followed by normothermia. Gene expression changes include activation of heat shock transcription factor 1 (HSF1), increased expression of heat shock proteins (HSP) and decreased expression and synthesis of other proteins, immune system activation via extracellular secretion of HSP. A cDNA microarray analysis found 140 transcripts to be up-regulated and 77 down-regulated in the cattle blood after heat treatment ($P < 0.05$). But still a comprehensive explanation for the direction of fold change and the specific genes involved in response to acute heat stress still remains to be explored. These findings may provide insights into the underlying mechanism of physiology of heat stress in cattle. Understanding the biology and mechanisms of heat stress is critical to developing approaches to ameliorate current production issues for improving animal performance and agriculture economics.

1. Introduction

Cattle, like other homeotherms, modulate internal body temperature by coupling the amount of heat produced through metabolism with the heat flow from the animal to its surrounding environment. Heat stress occurs as a result of an imbalance between heat production within the body and its dissipation (Kumar et al., 2011). Heat stressed cattle experience reduction in food intake, growth, immunity, milk yield and reproduction efficiency (Hahn, 1999). Environmental-induced hyperthermia jeopardizes animal welfare. The welfare of dairy cows can be evaluated on the basis of the temperature humidity index (THI) values. This index is usually used for evaluating the degree of stress on dairy cattle caused by weather conditions (Hahn and Mader, 1997), as it comprises the effects of both ambient temperature and relative humidity in an index. In the critical range of THI of 70–72, performance of dairy cattle declines and cooling becomes desirable. At THI of 72–78, milk production is seriously affected. In the dangerous category at THI of 78–82, the performance is severely affected and cooling becomes essential (Huber, 1996).

The detrimental effects of heat stress on animal welfare will likely become more of an issue if earth's climate continues to warm as predicted (IPCC, 2007). According to the IPCC fourth assessment report, a warming of up to 2 °C above 1990–2000 levels would have significant impacts on many unique and vulnerable systems. Changes in extreme temperature will accompany continued global warming. Thus raising the question how organisms adapt to extreme temperature events.

In the present investigation we sought to evaluate the changes occurring in genome-scale gene expression profile of the Indian zebu cattle (Sahiwal) during hyperthermia followed by normothermia. We used microarray-based global gene expression profiling as a high-resolution assay to understand the unique traits in cattle which make them resilient to climate change. Microarray technology is a powerful tool for seeking insights into gene regulation and its significance for biological function. However, our understanding of such relationships during environmental stress remains fragmentary.

The rationale behind the current study was to build a high confidence dataset of the differentially expressed genes of the peripheral circulation during the stage of heat stress and recovery as an initial step

Abbreviations: mRNA, messenger RNA; HSF1, heat shock transcription factor 1; HSPs, heat shock proteins; cDNA, complementary DNA; THI, temperature humidity index; IPCC, Intergovernmental Panel Climate Change; CCR, climate-controlled room; RT, rectal temperature; RR, respiration rates; IVT, in-vitro transcription; aRNA, amplified RNA; ANOVA, analysis of variance; MAS3, Molecule Annotation System 3; GO, gene ontology.

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towards their targeted validation. These genes once fully characterized would contribute to our understanding of the mechanisms implicit to the adaptability of zebu cattle i.e. regulation of their body temperature during heat stress and for maintenance of cellular function when hyperthermia ensues.

2. Materials and methods

2.1. Animals and experimental design

All the experimental procedures involving animals were approved by the Institutional Animal Ethics Committee (IAEC) at the National Dairy Research Institute (NDRI), Karnal. The heifers were managed under open housing system and were fed as per standard management practices followed at the institute farm. The feed and water were offered adlib. All the animals were apparently healthy at the time of experiments and had no pyrogenic response as revealed from their rectal temperature.

2.2. Heat treatment and sampling

The Sahiwal cattle ($n = 4$) were housed in the climate-controlled room (CCR) at NDRI, Karnal, which is equipped with an electric duct heater and a humidifier capable of maintaining air temperature and moisture. For the current study the CCR was maintained at 42 °C with 90% humidity and the cattle were exposed to heat stress conditions (42 °C temperature and 90% humidity; Temperature Humidity Index (THI) > 104) for a time period of 4 h and then recovered at control temperature (37 °C and 45% humidity) for 24 h and 48 h. The blood samples were drawn from the jugular vein in PAXgene blood RNA tubes immediately after 4 h of heat stress (heat stress condition) and before heat treatment (control condition), during 24 h and 48 h of recovery period. The blood samples collected in tubes were further processed for Microarray Expression Studies.

2.3. Determination of rectal and body surface temperature and respiration rate

The body surface (head, dorsal, ventral, rump) and rectal temperatures of the Sahiwal cattle were recorded before and after heat exposure using a gun Type Infrared Thermometer (Waco Mt-6, Kolkata, West Bengal, India) as shown in Table 1. Rectal temperature (RT) was recorded by inserting a digital clinical thermometer into the rectum for 1 min and care was taken to keep the thermometer bulb in close contact with the rectal mucosa. Respiration rates (RR) were recorded by counting the flank movements from a distance prior to examining the rectal temperature in order to avoid any disturbance to the animals.

2.4. RNA extraction, cDNA labeling & microarray hybridization

The blood samples were processed for RNA target preparation and microarray expression analysis using the GeneChip® 3′ IVT Express Kit (iLife Discoveries, India) according to manufacturer's instructions. The kit is based upon linear RNA amplification and employs T7 in-vitro transcription technology. It is also known as the Eberwine or reverse transcription-IVT (RT-IVT) method. RT-IVT was experimentally validated using TaqMan®RT-PCR. In the GeneChip®3′ IVT Express Protocol total RNA undergoes reverse transcription to synthesize first-strand cDNA. First-Strand cDNA is primed with T7 oligo(dT) primer to synthesize cDNA containing a T7 promoter sequence. This cDNA was then converted into a double-stranded DNA template for transcription. The reaction employs DNA polymerase and RNase H to simultaneously degrade the RNA and synthesize second-strand cDNA. In-vitro transcription synthesized Biotin-Modified aRNA (amplified RNA) with IVT Labeling Master Mix that generated multiple copies of biotin-modified aRNA from the double-stranded cDNA templates; this is the amplification step. The aRNA was then purified to remove unincorporated NTPs, salts, enzymes, and inorganic phosphate. Fragmentation of the biotin-labeled aRNA prepares the sample for hybridization onto GeneChip 3′ expression arrays.

Table 1
Tabulated summary of physiological parameters recorded after heat treatment and control condition. Values are indicated as mean ± SEM. Values with different superscript in the same row are statistically significant ($P < 0.05$).

Parameters	0 h (control)	4 hour heat treatment	24 hour recovery
Rectal temperature	103.2 ± 0.07414^{a}	105.3 ± 0.2499^{b}	103.3 ± 0.1853^{c}
Forehead	43 ± 1.578^{a}	39.53 ± 1.359^{b}	39.53 ± 1.359^{b}
Dorsal	43.12 ± 1.706^{a}	39.03 ± 0.7532	40.17 ± 0.7111^{c}
Ventral	41.07 ± 1.77	37.65 ± 0.6435	39.12 ± 0.5573
Rump	42.83 ± 2.155	38.88 ± 0.7307	40.28 ± 1.073
Respiration rate	20 ± 1.414^{a}	41 ± 1.732^{b}	28 ± 0.8165^{c}

2.5. Data analysis

Raw signal intensity data generated from the samples (in form of .CEL files) was imported into GeneSpring software. The data was background corrected using MAS5 algorithm to reduce background noise, adjusted for cross hybridization and adjusted to estimate expression values on proper scale. Background corrected data was further summarized (baseline to median of all samples) using MAS5 algorithm. Linearly transformed data was normalized (normal scaling) to reduce variations due to non-biological factors in order to compare data across multiple chips reliably. Normalized data was analyzed for statistically significant gene expression differences between control and treatment groups using One-Way ANOVA according to the Fc (Fold change) statistic that uses overall gene expression variation to calculate a gene-specific variance. For the one-way ANOVA a null distribution was generated using 1000 permutations to account for the unequal variance and non-normal distributions of the response variables and a P-value of 0.05 was used instead of using a multiple-comparison correction, which was deemed too strict. To keep the number of false positives to alpha = 0.05, a false discovery rate adjustment was used (Reiner et al., 2003). Genes that were statistically significant in expression between heat stress and control groups in at least one time point were grouped according to fold expression (up- vs. down-regulation). The data was further clustered using Hierarchical Clustering based on entities (Genes) and conditions (Treatment).

3. Results and discussion

As per Hans Selye, stress is the nonspecific response of the body to any demand, whether it is caused by, or results in, pleasant or unpleasant conditions. Stressors are adverse forces that can be emotional or physical. Both the magnitude and chronicity of stressors are important for eliciting a response(s) (George, 2009). Primarily there are two types of stressors, psychosocial stressor and biogenic stressors e.g. caffeine, exercise and heat (Everly and Lating, 2002). In the present study on Sahiwal cattle, we used cDNA microarrays to describe gene expression changes due to heat exposure and during recovery from heat stress in peripheral blood mononuclear cells of the Sahiwal cattle. Peripheral blood is an accessible and informative source of transcriptional information for many metabolic changes, immune response functions and other studies. There can be significant advantages to analyzing RNA isolated from whole blood, particularly in nonclinical and clinical studies (Kristina et al., 2009). Gene expression levels of Sahiwal cattle were studied after exposure at 42 °C and 90–95% relative humidity over a 4 h period and during recovery up to 48 h in exposed and controls. Relative humidity of 95% and 42 °C temperature on THI corresponds to extreme danger zone. Within certain THI range, normal thermoregulatory mechanisms are capable of restoring to 37 °C from either hyperthermia or hypothermia. However, if temperature rises above 42.5 °C, then metabolic pathways may be so affected that inappropriate physiological