Cardiac adaptive mechanisms of Tibetan antelope (*Pantholops hodgsonii*) at high altitudes

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Objective—To identify cardiac mechanisms that contribute to adaptation to high altitudes in Tibetan antelope (*Pantholops hodgsonii*).

Animals—9 male Tibetan antelope and 10 male Tibetan sheep (*Ovis aries*).

Procedures—Tibetan antelope and Tibetan sheep inhabiting a region with an altitude of 4,300 m were captured, and several cardiac variables were measured. Expression of genes for atrial natriuretic peptide, brain natriuretic peptide, and calcium-calmodulin–dependent protein kinase II δ was measured via real-time PCR assay.

Results—Ratios of heart weight to body weight for Tibetan antelope were significantly greater than those of Tibetan sheep, but ratios of right-left ventricular weights were similar. Mean ± SD baseline heart rate (26.33 ± 6.15 beats/min) and systolic arterial blood pressure (97.75 ± 9.56 mm Hg) of antelope were significantly lower than those of sheep (34.20 ± 6.57 beats/min and 130.06 ± 17.79 mm Hg, respectively). The maximum rate of rise in ventricular pressure in antelope was similar to that in Tibetan sheep, but after exposure to air providing a fraction of inspired oxygen of 14.6% or 12.5% (ie, hypoxic conditions), the maximum rate of rise in ventricular pressure of the antelope increased significantly to 145.1% or 148.1%, respectively, whereas that of the sheep decreased to 68.4% or 70.5%, respectively. Gene expression of calcium-calmodulin–dependent protein kinase II δ and atrial natriuretic peptide, but not brain natriuretic peptide, in the left ventricle of the heart was significantly higher in antelope than in sheep.

Conclusions and Clinical Relevance—Hearts of the Tibetan antelope in this study were well adapted to high-altitude hypoxia as shown by higher heart weight ratios, cardiac contractility in hypoxic conditions, and expression of key genes regulating cardiac contractility and cardiac hypertrophy, compared with values for Tibetan sheep. (*Am J Vet Res* 2012;73:809–813)

The Qinghai-Tibetan Plateau is the highest and biggest plateau in the world, with an area of 2.5 million km^2 and a mean altitude > 4,500 m. The flora and fauna on the plateau are constantly exposed to a harsh environment with a low percentage of atmospheric oxygen, low temperature, and high amount of solar radiation, all of which challenge the inhabitants' reproductive success. Hypoxia attributable to the high altitude is the most important ecological factor restricting the viability of the humans and other animals that live there.

Native animal species have survived on the plateau for thousands of years and have developed their own mechanisms of adaptation to harsh environmental stress.[1] Elucidation of the mechanisms by which native animals have adapted to these conditions should shed

Abbreviations

+dp/dt	Maximum rate of left ventricular pressure increase in systole
–dp/dt	Maximum rate of left ventricular pressure decrease in diastole
ANP	Atrial natriuretic peptide
BNP	Brain natriuretic peptide
CaMKII	Calcium-calmodulin–dependent protein kinase II
DAP	Diastolic arterial blood pressure
F_{IO_2}	Fraction of inspired oxygen
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
Hb	Hemoglobin
HIF	Hypoxia-inducible factor
HR	Heart rate
LV	Left ventricle
RV	Right ventricle
SAP	Systolic arterial blood pressure

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light on the pathological responses of nonadapted animals from the lowlands, which develop high-altitude mountain sickness and chronic pulmonary hypertension upon short and prolonged exposure to high altitudes.

Tibetan antelope (*Pantholops hodgsonii*) are native mammals that have adapted to the Qinghai-Tibetan Plateau. Tibetan antelope live on the high mountain steppes and semidesert areas of the plateau at altitudes of 3,700 to 5,500 m. In the same environmental conditions, Tibetan sheep (*Ovis aries*) roam the highlands of the plateau at a mean altitude of 3,000 m. The purpose of the study reported here was to identify cardiac adaptive mechanisms in Tibetan antelope through characterization of LV contractility, heart morphology, and expression of cardiac-related genes in Tibetan antelope and Tibetan sheep.

Materials and Methods

Animals—Nine male Tibetan antelope and 10 male Tibetan sheep were captured from Kekexili Natural Reservation (altitude, 4,300 m) and transported to Gurmud (altitude, 2,800 m), where the study was conducted immediately after the arrival of the animals. The study protocol was approved by the State Forestry Administration and complied with the Guide for the Care and Use of Laboratory Animals by the Ministry of Science and Technology of the People's Republic of China.

Physiologic and hemodynamic measurements—Animals were weighed and then anesthetized via IP injection of xylazole hydrochloride (1 to 5 mg/kg) and 500 U of heparin. For each animal, a 5-mL blood sample was collected via jugular venipuncture with a 16-gauge needle into disposable syringes and transferred into a sterile test tube with anticoagulant (tripotassium EDTA). The blood samples were analyzed for RBC count, Hb concentration, WBC count, and Hct with a hematologic analyzer.[a]

The neck of each animal was shaved with a clipper blade, and the shaved area was disinfected with 0.5% iodophor solution. Cardiac catheterization was subsequently performed, with the catheter inserted into the LV via the right carotid artery. When animals recovered consciousness, a data acquisition system[b] connected to the catheter was used to measure HR, SAP, DAP, +dp/dt, and –dp/dt. These data were analyzed with computer software.[c] After 20 minutes of stabilization, the animals were exposed to air containing a low concentration of oxygen through use of a venting system involving a 1-way-valve mask to provide an F_{IO_2} ranging from 12.5% to 14.6%. The same measurements were once again obtained.

Heart measurements—After cardiac catheterization measurements were complete, 5 antelope and 5 sheep were euthanized with sodium pentobarbital (200 mg/kg, IV), and their hearts were removed for evaluation. The remaining 4 antelope and 5 sheep were released back into the reservation after they had recovered from testing. Excess blood was removed from each heart with tissue paper, and each heart was weighed. The atria were excised, and the free walls of the RV and LV and the septum were dissected and weighed separately. Cardiac and RV hypertrophy were assessed by calculating the ratio of heart weight to body weight and of RV weight to LV plus septum weight.

Gene expression measurement—The hearts were cut at midpapillary level, and the tissue pieces were snap frozen in liquid nitrogen. Total RNA was extracted from the tissues by use of a reagent[d] following a standard protocol and used as the template for reverse transcription in a total volume of 20 µL with 200 U of Moloney murine leukemia virus reverse transcriptase.[e] The synthesized cDNA was amplified via PCR assay with specific primers (**Appendix**). The general PCR protocol included predenaturation at 94°C for 3 minutes and final extension at 72°C for 10 minutes, but the cycles differed by gene as follows: CaMKIIδ (94°C for 30 seconds, 56°C for 60 seconds, and 72°C for 1.5 minutes), ANP (94°C for 30 seconds, 58°C for 50 seconds, and 72°C for 1.5 minutes), BNP (94°C for 30 seconds, 60°C for 50 seconds, and 72°C for 1.5 minutes), β-actin (94°C for 30 seconds, 56°C for 60 seconds, and 72°C for 1.5 minutes), and GAPDH (94°C for 30 seconds, 56°C for 100 seconds, and 72°C for 1.5 minutes).

The PCR products were separated on a 1.2% agarose electrophoretic gel, purified with a kit[f] in accordance with the manufacturer's instructions, and then cloned[g] following a standard protocol. Because the sequences of cardiac-related genes were not available in the National Center for Biotechnology Information database, these genes were amplified by reverse transcriptase PCR and cloned[g] for DNA sequencing. The sequences of the PCR product were determined with a DNA analyzer.[h] A sequence homology search was performed with a bioinformatics search tool.[i]

Real-time PCR assay—Real-time PCR analysis was performed with a real-time PCR system[j] in a 25-µL reaction volume containing 12.5 µL of premix,[k] 1 µL of primer (10 µmol/L), and 1 µL of cDNA templates. All samples were prepared as 1:10, 1:100, and 1:1,000 dilutions, and each reaction at each dilution was evaluated performed in triplicate. Results were analyzed with computer software.[l]

Statistical analysis—All data are reported as mean ± SD. Statistical analysis was performed with statistical software.[m] Mean values were compared between antelope and sheep by performance of the independent samples Student *t* test, and values of $P < 0.05$ were considered significant.

Results

Animals—The mean ± SD body weight of the 9 Tibetan antelope was 37.96 ± 9.45 kg, which did not differ significantly from that of the 10 Tibetan sheep (37.67 ± 4.68 kg).

Hematologic values—Blood Hb concentration (12.23 ± 1.32 g/dL) and Hct (35.62 ± 6.72%) in the antelope were significantly ($P < 0.01$) lower than in the sheep (14.13 ± 1.05 g/dL and 48.90 ± 9.75%, respectively). However, WBC and RBC counts did not differ between antelope (6.55 ± 1.60 × 10^9 cells/L and 8.29 ± 0.95 × 10^{12} cells/L, respectively) and sheep (5.67

± 1.93 × 10^9 cells/L and 8.81 ± 2.76 × 10^{12} cells/L, respectively).

Hemodynamic measurements—Before induction of hypoxic conditions (ie, at baseline), HR, SAP, and DAP in the antelope were 26.33 ± 6.15 beats/min, 97.75 ± 9.56 mm Hg, and 69.53 ± 7.55 mm Hg, respectively, whereas in sheep, they were 34.20 ± 6.57 beats/min, 130.06 ± 17.79 mm Hg, and 91.70 ± 13.58 mm Hg, respectively. The difference between groups was significant ($P < 0.05$) for all variables, with lower values detected in antelope. However, the maximum rate of LV pressure change did not differ significantly ($P > 0.05$) between antelope (+dp/dt, 633.87 ± 159.49 mm Hg/s; –dp/dt, –396.93 ± 166.68 mm Hg/s) and sheep (+dp/dt, 564.76 ± 229.03 mm Hg/s; –dp/dt, –259.82 ± 124.21 mm Hg/s).

After reduction of the F_{IO_2} from 21.1% to 14.6% and 12.5% (which was used to simulate altitudes of 5,300 and 6,300 m, respectively), SAP, DAP, and –dp/dt in Tibetan antelope were similar to the values at baseline. In Tibetan sheep, SAP and DAP decreased significantly, although the –dp/dt changed little (**Figure 1**). On the other hand, the +dp/dt increased significantly in the antelope to 145.1% and 148.1%, respectively, whereas it decreased in the sheep to 68.4% and 70.5%, respectively.

Heart weight and RV hypertrophy—The heart weight–to–body weight ratio of Tibetan antelope (8.38 ± 0.30%) was significantly greater than that of Tibetan sheep (4.84 ± 0.52%), but the RV–to–LV plus septum weight ratio was essentially the same (0.34 ± 0.23% and 0.36 ± 0.49%, respectively).

Cardiac-associated genes—The partial sequences for the cardiac-associated genes as well as for GAPDH as an internal control for quantitative real-time PCR analysis were accepted by GenBank for Tibetan antelope and Tibetan sheep, respectively, as follows: CaMKII (accession Nos. HQ230328 and HQ449191), ANP (accession Nos. HQ449188 and HQ449190), BNP (accession Nos. HQ449187 and HQ449189), and GAPDH (accession Nos. HQ625519 and HQ625518).

Expression of hypertrophy-associated genes—Quantitative real-time PCR analysis revealed that the cycle threshold values of GAPDH were similar in various samples, indicating that GAPDH was the proper choice as an internal control. The GAPDH was consequently used to normalize expression of the genes for CaMKIIδ, ANP, and BNP in the LV of the heart. Gene expression of CaMKIIδ and ANP (1.347 ± 0.219 and 1.057 ± 0.175, respectively) was significantly ($P < 0.01$) higher in Tibetan antelope than in Tibetan sheep (0.545

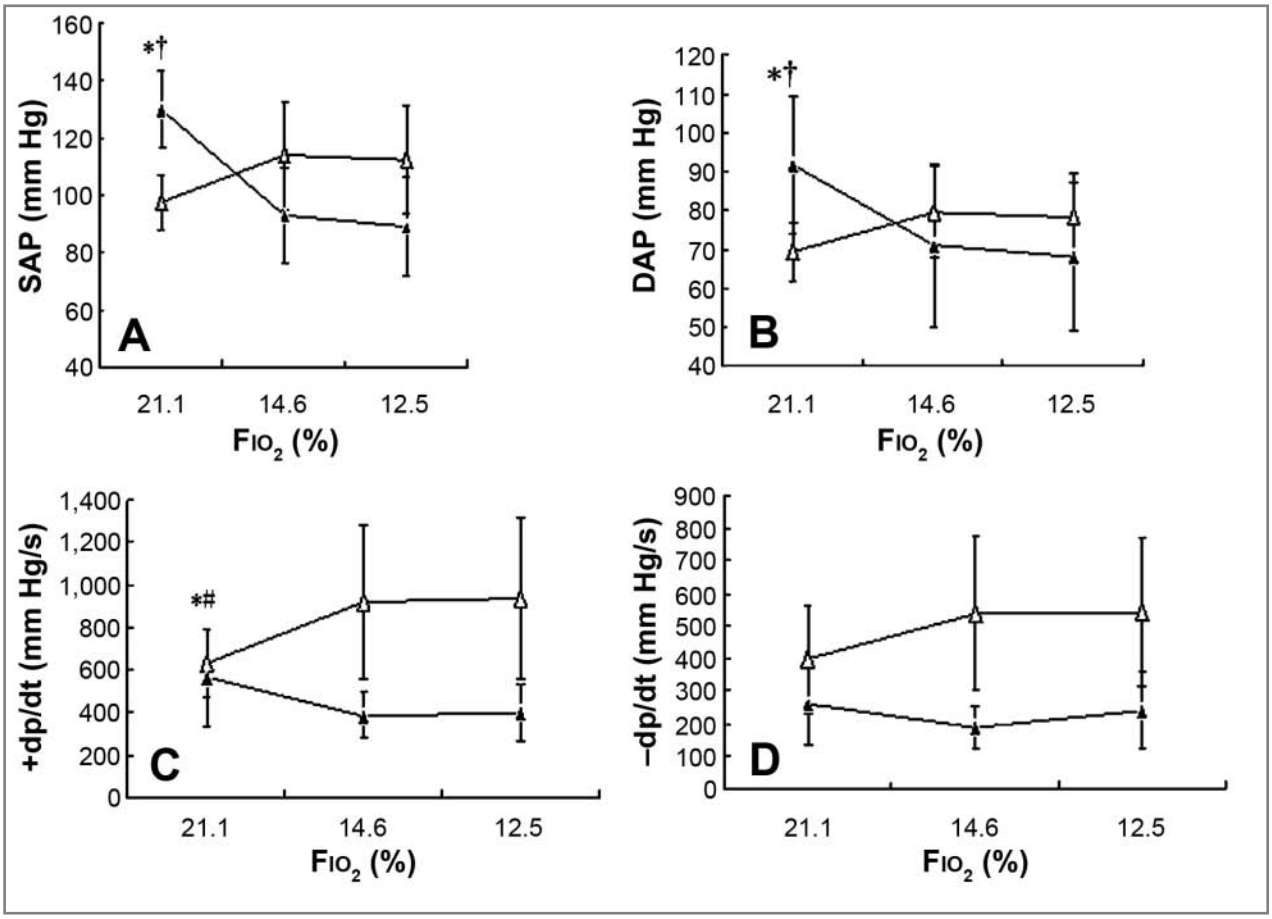

Figure 1—Mean ± SD SAP (A), DAP (B), +dp/dt (C), and –dp/dt (D) before (F_{IO_2}, 21.1%) and after exposure to air with lower oxygen content (F_{IO_2} of 14.6% or F_{IO_2} of 12.5%) in Tibetan antelope (*Pantholops hodgsonii*; n = 9; white triangles) and Tibetan sheep (*Ovis aries*; 10; black triangles). *Indicated sheep value is significantly ($P < 0.05$) different from that for an F_{IO_2} of 14.6% or 12.5%. †Indicated antelope value is significantly ($P < 0.05$) different from the corresponding sheep value. #Value is significantly ($P < 0.05$) different from F_{IO_2} of 14.6% or 12.5% in Tibetan antelope.

± 0.002 and 0.436 ± 0.161, respectively), but expression of BNP was not significantly different (antelope, 1.554 ± 0.134; sheep, 1.127 ± 0.357).

Discussion

The present study was designed to characterize cardiac adaptation to a high altitude in Tibetan antelope. Findings indicated the ratio of heart weight to body weight in Tibetan antelope was significantly greater than that of Tibetan sheep, although the 2 species had similar values for the ratio of RV weight to LV plus septum weight. Thus, it appeared that the antelope had myocardial hypertrophy, which can be classified as physiologic or pathological.[2] Physiologic hypertrophy is an adaptive response that maintains cardiac function by increasing the size of cardiomyocytes and thus enhancing hemodynamic loads in physiologic conditions such as postnatal developmental growth, exercise, and hypoxia.[3] Therefore, we propose that the hearts of Tibetan antelope may adapt to high altitudes by physiologic hypertrophy.

A simple and reliable index of ventricular contractility is +dp/dt, which is useful irrespective of ventricular morphology, localized wall motion abnormalities, or structural abnormalities.[4] It can be used to reflect the change in contractility assessed by load-independent indices in both acute and chronic exposures.[5,6] In nonhypoxic conditions, the HR and SAP of Tibetan antelope were significantly lower than those of Tibetan sheep. On the other hand, the +dp/dt in the antelope was similar to that of the sheep in the same conditions. However, after exposure to hypoxic conditions (Fio_2 of 14.6% or 12.5%) that simulated an altitude of 5,300 or 6,300 m, +dp/dt of Tibetan antelope increased significantly, suggesting that the antelope adapted to high-altitude hypoxia by lowering HR, SAP, and heart contractility, thereby minimizing oxygen consumption. A decrease in sympathetic tone and increase in parasympathetic tone may be responsible for these changes after long-term exposure to high-altitude hypoxia.[7]

When Tibetan antelope were under hypoxic stress, the increase in +dp/dt contributed to oxygen delivery to tissues. However, +dp/dt of Tibetan sheep decreased in hypoxic conditions, which suggested that LV function of Tibetan sheep became depressed by severe hypoxia, even though both species live on the Qinghai-Tibetan Plateau. It therefore appears that the hearts of Tibetan antelope are better adapted to high-altitude hypoxia.

To investigate the potential molecular mechanisms underlying the cardiac adaptation of Tibetan antelope to high altitudes, we cloned genes related to cardiac function (CaMKIIδ, ANP, and BNP) from Tibetan antelope and Tibetan sheep and then compared their degrees of expression. Calcium-calmodulin–dependent protein kinase II is a ubiquitous mediator in calcium signaling and modulates calcium handling and physiologic processes in cardiomyocytes such as excitation-contraction coupling, gene transcription, and apoptosis.[8,9] In addition, the CaMKII gene was recently identified as 1 of 10 candidate genes associated with adaptation to high-altitude hypoxia.[10] Among the 4 CaMKII isoforms (α, β, δ, and γ),[11] CaMKIIδ is predominant in the heart.[12,13]

Atrial natriuretic peptide and BNP are cardiac hormones as well as markers of cardiac hypertrophy and congestive heart failure, and the secretion of ANP is regulated by intracellular Ca^{2+} via the ryanodine receptor and CaMKII.[14] The quantitative real-time PCR analysis used in the present study revealed a higher degree of expression of CaMKII and ANP genes but not the BNP gene in Tibetan antelope, which is consistent with previous reports that expression of CaMKII mRNA increases in response to chronic hypoxia[15] and that CaMKII activity is positively correlated with LV ejection fraction and cardiac index.[16] Furthermore, the increase in expression of CaMKIIδ mRNA was accompanied by an increase in expression of ANP mRNA, which may contribute to cardiac physiologic hypertrophy to maintain calcium homeostasis and ventricular contraction in Tibetan antelope. The unchanged expression of BNP suggested that there is no cardiac dysfunction in these antelopes' hearts. Taken together, the data may indicate that physiologic hypertrophy helps improve cardiac contractility and output to deliver oxygen to tissues at high altitudes.

Results of the present study indicated that blood Hb concentration and Hct were significantly lower in Tibetan antelope than in Tibetan sheep. This is consistent with results of human studies[17–19] that showed that Tibetans who reside at high altitudes have a lower Hb concentration, decreased arterial O_2 content, and lack of hypoxic pulmonary vasoconstriction, compared with other Tibetans. Although the mechanisms of these differences are not fully understood, data[10,20,21] exist to suggest the genes in the HIF oxygen-signaling pathway have been subject to highly positive selection in Tibetans. Indeed, the genes *EPAS1* (HIF2α), *EGLN1* (a regulator of HIF), and *PPARA* (a transcriptional target of HIF) are strongly associated with reduced Hb concentrations,[10,20,21] providing additional evidence of genetic adaptation in residents of high-altitude environments.

The present study provided the first evidence that Tibetan antelope as native animals on the Qinghai-Tibetan Plateau have developed special cardiac characteristics during adaptation to high-altitude hypoxia, as evidenced by an increase in heart weight, cardiac contractility, and expression of key genes regulating cardiac contractility and cardiac hypertrophy. Additional investigation of the mechanisms underlying cardiac adaptations in Tibetan antelope will help our understanding of chronic high-altitude mountain sickness in animals and help us to pursue potential prevention and treatment measures.

a. BC-2300 Hematology Analyzer, Mindray, Shenzhen, People's Republic of China.
b. MP100 series, BIOPAC Systems Inc, Goleta, Calif.
c. Acqknowledge, version 3.5.2, BIOPAC Systems Inc, Goleta, Calif.
d. TRIzol, Invitrogen, Shanghai, China.
e. Tiandz Inc, Beijing, China.
f. Column DNAback kit, Tiandz Inc, Beijing, China.
g. pGEM-T Vector System, Promega Corp, Madison, Wis.
h. ABI 3730 DNA Analyzer, Invitrogen, Shanghai, China.
i. BLAST, National Center for Biotechnology Information, National Institutes of Health, Bethesda, Md. Available at: blast.ncbi.nlm.nih.gov/. Accessed Oct 22, 2010.

j. iQ5 Gradient Real-Time PCR System, Bio-Rad Laboratories Inc, Berkeley, Calif.
k. 2xSYBR Green Master Mix, Applied Biosystems, Foster City, Calif.
l. iQ5 Optical System Software, Bio-Rad Laboratories Inc, Berkeley, Calif.
m. Statistical Package for the Social Sciences, version 13.0, SPSS Inc, Chicago, Ill.

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Appendix

Primer sequences and size of PCR products used to measure cardiac gene expression.

Gene	Primer	Sequence (5′ to 3′)	Primer length (bp)	Product length (bp)
CaMKIIδ	CaMKIIδ-F	CATGTGCACCTGGTGGGCGA	20	137
	CaMKIIδ-R	TGCCACTTTCCATCCCGGCG	20	—
ANP	ANP-F	CATCTTCTGCTGCTTCCTCA	20	152
	ANP-R	GCCTTTGTCCGTCTCTGTCT	20	—
BNP	BNP-F	GAGAGCCCCCGTCCCACAGGT	21	153
	BNP-R	CCTCCCAAAGCAGCCAGACCC	21	—
GAPDH	GAPDH-F	CTGAGTATGTGGTGGAGTC	20	175
	GAPDH-R	ATCTTGAGGGTGTTGTTAT	20	—

— = Not applicable.