ORIGINAL RESEARCH

Plasma proANP₁₋₉₈ Response During High Altitude Stress: Effect of Age and Ethnicity

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Objective.—Acclimatization to high altitude (HA) is accompanied by decrease in plasma atrial natriuretic peptide (ANP). On the other hand, circulating levels of the hormone are known to be influenced by age and ethnicity. The impact of these factors on ANP response during prolonged HA exposure remains unexplored. Hence, this study was conducted to examine possible age and ethnic variation in plasma proANP₁₋₉₈ levels in men after 3 to 4 weeks at HA.

Methods.—Lowlanders (LL) were studied at sea level (SL) and after 3 to 4 weeks at an altitude of ~4500 m. The LL group comprised Rajput ($n = 48$), Gorkha ($n = 40$), and South Indian ($n = 43$) ethnicities. Another group of HA natives (Ladakhi, $n = 40$) were studied at ~4500 m only. Subjects were between 20 and 50 years of age. Estimation of plasma proANP₁₋₉₈ and biochemical, hematologic, and physiologic evaluation was done.

Results.—In LL at HA, proANP₁₋₉₈ levels decreased ($P < .001$); plasma arginine vasopressin decreased ($P < .05$ in Rajputs and South Indians); and total protein, hemoglobin, and hematocrit increased ($P < .05$). Heart rate increased ($P < .05$), whereas arterial oxygen saturation decreased ($P < .05$) in all LL at HA. Ethnicity but not age variation in proANP₁₋₉₈ was observed under HA stress. In HA natives, plasma proANP₁₋₉₈ was higher than LL at HA and did not exhibit any age variation.

Conclusions.—Plasma proANP₁₋₉₈ levels, reflecting medium-term ANP secretion, decrease during prolonged exposure to HA in LL. This is due to diuresis leading to plasma volume reduction that occurs during the acclimatization process. Ethnicity but not age variation is associated with plasma proANP₁₋₉₈ under HA stress.

Key words: proatrial natriuretic peptide, men, chronic hypoxia, high altitude

Introduction

Hypoxia is a known secretagogue for atrial natriuretic peptide (ANP) and enhances ANP gene expression in mammals.¹ Atrial natriuretic peptide is primarily secreted from atrial cardiomyocytes.² Cleavage of proANP (1-126), the predominant storage form of ANP in atrial granules, releases equimolar amounts of the biologically active peptide ANP (99-126) and proANP (1-98), the major circulating N-terminal ANP (proANP₁₋₉₈), into the circulation.³ For diagnostic accuracy and prognostic relevance, estimation of plasma proANP₁₋₉₈ has been suggested as a good reliable measure to measure of ANP as it is more stable ex vivo, has a longer plasma half-life,

and, consequently, higher plasma concentrations (10 to 50 times) than ANP.² Hence, proANP₁₋₉₈ is more likely to reflect medium-term ANP secretion. Atrial natriuretic peptide release during hypoxia plays an important role in decreasing pulmonary arterial pressure (PAP), increasing fluid shift out of the circulation, and facilitating action of erythropoietin.⁴ However, subjects acclimating normally have little or no change in ANP, and it has been suggested that ANP may be triggered in pathologic conditions in which natriuresis is impaired.⁵⁻⁷ In high altitude natives (HANs), Antezana et al⁸ have observed that plasma ANP level was less than that in sea level (SL) natives despite high PAP. However, ANP level in Lada-khis who are HANs of the Himalayas is not known, and it has been reported that pulmonary circulation in the Himalayan people is well adapted and better than that of the Andean people.⁹

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Apart from various environmental and psychological factors, levels of plasma hormones are also influenced by age and exhibit ethnic variation. This may also affect the response to stress. The Framingham Study was the first to identify that plasma natriuretic peptide levels exhibit heritability and genetic linkage along with age variations in humans.¹⁰ Age-associated increase in plasma ANP and proANP₁₋₉₈ has been well documented.^{2,10} Furthermore, aging-associated physiologic alterations in factors affecting ANP release such as PAP in hypoxia have been observed in both humans¹¹ and animals.¹² Some studies have reported the association between ANP gene polymorphisms and plasma ANP levels/proANP₁₋₉₈ levels, hypertension, cardiac hypertrophy, proteinuria, stroke, and recurrent stroke.^{10,13} In this perspective, it is possible that variations in proANP₁₋₉₈ levels in men from different ethnic background across various age groups may be different under high altitude (HA) stress thereby modulating the adaptive processes. Also, studies concerning the influence of age on chronic hypoxia-induced structural and functional alterations in the cardiovascular system are very few and controversial.¹⁴ With respect to the Indian population, age and ethnic variations in proANP₁₋₉₈ levels under HA stress have not been studied previously. This population is characterized by high levels of endogamy and a high degree of genetic differentiation among various ethnic groups.¹⁵ The purpose of this report was to explore these issues by presenting data on morning plasma proANP₁₋₉₈ levels in lowlanders of different age groups and ethnicities at HA as compared with that at SL and to compare proANP₁₋₉₈ response in lowlanders with that of HANs at HA.

Materials and methods

SUBJECTS

Healthy male subjects from the armed forces ($n = 171$) between 20 and 50 years of age were enrolled for the study after informed consent and approval from the ethics committee of the institute. Subjects were chosen from four ethnically distinct groups based on geographic zones and morphologic types¹⁶: Rajput, Gorkha, South Indian, and Ladakhi. Rajputs are from northwestern India (Rajasthan) with Caucasoid features; Gorkhas are basically from submountainous regions (2000 to 3000 m) from northern India with Mongoloid features, and they had been living at SL for ~4 years prior to the study; and South Indians are people from the southern plains and coastal areas of the country with Australoid features. Ladakhis are HANs from Ladakh of the Himalayas, which is located with the Karakoram to the northwest, the Himalayas in the southwest, and the Trans-Himala-

yas at its core. High-altitude natives living at HA had never been to SL. The subjects of Rajput, Gorkha, and South Indian ethnicities are referred to as lowlanders. Subjects were categorically divided into 4 age groups (years): 20 to 24, 25 to 29, 30 to 34, and 35 to 50.

STUDY PROTOCOL

All subjects of lowland origin were evaluated at SL. These subjects were inducted to an altitude of 3500 m where they stayed for 7 days. Then, they were inducted to a higher altitude: ~4500 m for Rajputs and South Indians and ~4400 m for Gorkhas, where they continued to stay. At this final altitude, the lowland subjects were evaluated after 3 to 4 weeks of stay. Another group of HANs were studied at ~4500 m only.

DIET

Diet consisted of authorized rations of the Indian Army that included an average intake of 13 819 kJ (carbohydrate 61%, lipid 26%, protein 13%) at SL and 14 657 kJ (carbohydrate 60%, lipid 28%, protein 12%) for HA operations.¹⁷ Food was consumed at the common mess at respective locations and is essentially the same for all subjects. Salt content in the ration was 14 to 19g.¹⁸ There was no fluid restriction during the study.

ANTHROPOMETRY

Body weight and height were measured using an electronic weighing machine (Delmer, India) and an anthropometer. Height was measured to the nearest 0.5 cm and weight to the nearest 0.5 kg. Body fat was estimated indirectly by calculating body mass index (BMI): body weight (kg) divided by the height squared (m^2).

SAMPLE ANALYSIS

In all the subjects, fasting venous blood samples were collected between 0700 and 0800 hours. The subjects were rested for half an hour in a sitting position and then for 5 minutes in a supine position before blood drawing from the antecubital vein. Samples collected in ethylenediamine tetraacetic acid (EDTA) tubes were centrifuged, and plasma was stored at $-80^{\circ}C$ until analysis.

BIOCHEMICAL PARAMETERS

Plasma proANP₁₋₉₈ level (nmol/L) was measured by enzyme-immunoassay method (Biomedica Gruppe, Austria; code BI-20892). This kit is suitable for the use of EDTA plasma. It is recommended for stability of the

Table 1. Characteristics of the study subjects

Variables	Lowlanders						HA native (HA only)
	Rajput		Gorkha		South Indian		
	SL	HA	SL	HA	SL	HA	
No. of subjects	48		40		43		40
Age (years)	31.4 ± 1.0		30.3 ± 0.9		30.8 ± 1.2		31.1 ± 1.2
Final altitude of study (m)	~4500		~4400		~4500		~4500
Barometric pressure (kPa)	60		62		60		60
Ambient temperature (°C)	−6 to 10		−6 to 10		3 to 22		3 to 22
Duration of stay at HA			22–28 days (mean 25 days)				Lifelong
Height (m)	1.74 ± 0.01		1.67 ± 0.01		1.72 ± 0.07		1.69 ± 0.01 ^{bc}
Weight (kg)	67.6 ± 1.1	64.8 ± 1.1 ^a	63.9 ± 1.2	61.9 ± 1.1 ^a	66.5 ± 1.0	64.9 ± 0.9 ^a	62.9 ± 1.3
BMI (kg/m ²)	22.3 ± 0.3	21.3 ± 1.0 ^a	22.9 ± 0.4	22.2 ± 0.3 ^a	22.6 ± 0.3	22.1 ± 0.3 ^a	22.0 ± 0.4

Values are expressed as mean ± SEM. Level of significance: $P < .05$.

SL, sea level; HA, high altitude. SL vs HA (^a); Rajput vs HA native (^b); South Indian vs HA native (^c).

peptide that sample storage be at -20°C or -70°C , and samples can be subjected to 4 freeze/thaw cycles without any loss of immune reactivity. The sensitivity of the assay was 0.050 nmol/L. The interassay and intra-assay coefficients of variation were 4% and 2%, respectively. The cross-reactivities with other natriuretic peptides were less than 1%.

Plasma arginine vasopressin (AVP) level (ng/mL) was measured by enzyme-immunoassay method (Phoneix Pharmaceuticals Inc., USA). The sensitivity of the assay was 0.06 ng/mL. The interassay and intra-assay coefficients of variation were <14% and <5%, respectively. Hormone estimations on all plasma samples were performed using the Molecular Devices VERSAmax Reader (Molecular Devices, USA). Plasma protein (g/dL) was measured by the Lowry method.¹⁹

HEMATOLOGIC PARAMETERS

Hemoglobin (g/dL) and hematocrit (%) was measured using the MS4 analyzer (Melet Schloesing, France).

PHYSIOLOGIC PARAMETERS

Heart rate (HR; beats/minute) and arterial oxygen saturation by pulse oximetry (SpO_2 ; %) were measured by the Multi Parameter Patient Monitor (5553Pro; BPL, India).

STATISTICAL ANALYSIS

Data were analyzed by General Linear Model (GLM) repeated measure using SPSS software to see the change in proANP₁₋₉₈ level due to age (factor 1), ethnicity (factor 2), and repeated over altitude (ie, at SL and HA).

To compare the three ethnicities (ie, lowlanders) with HANs (ie, Ladakhi) at HA for all age groups, GLM analysis was performed. Post hoc analysis was done using Student-Newman-Keuls test and Dunnett test for the two hypotheses, respectively. For other variables, data were analyzed using paired t test to compare SL with HA. P value less than .05 was considered significant. Values are expressed as mean ± SEM.

Results

The baseline characteristics of the study subjects are as shown in Table 1. At HA, body weight and BMI showed a significant decrease ($P < .05$) in lowland ethnicities compared with those at SL. There was no ethnic variation in BMI. In lowlanders, hemoglobin, hematocrit, and plasma protein showed a significant ($P < .05$) increase compared with those at SL. Plasma AVP showed a significant ($P < .05$) decrease in all lowland ethnic groups at HA. Significant ($P < .05$) changes in HR (increase) and SpO_2 (decrease) were observed in lowlanders at HA. These data are shown in Table 2. Observations on plasma proANP₁₋₉₈ levels are as follows (Table 3):

Sea level: Plasma proANP₁₋₉₈ levels in lowlanders were in the low normal range. Ethnicity ($P < .001$) but not age variation in plasma proANP₁₋₉₈ was observed among lowlanders. Plasma level in South Indians was higher than that in Rajputs and Gorkhas. There was no difference in plasma levels between Rajputs and Gorkhas.

High altitude: Changes observed in plasma proANP₁₋₉₈ in lowlanders were in the low normal range of SL values. In comparison with those at SL, plasma levels showed a significant ($P < .001$) decrease in all low-

Table 2. Biochemical, hematologic, and physiologic characteristics for each ethnic group in subjects between 20 and 50 years of age

Variables	Lowlanders						HA native (HA only)
	Rajput		Gorkha		South Indian		
	SL	HA	SL	HA	SL	HA	
Plasma protein (g/dL)	7.2 ± 0.1	7.7 ± 0.1 ^a	7.6 ± 0.1	8.2 ± 0.1 ^a	7.7 ± 0.2	8.9 ± 0.1 ^a	8.0 ± 0.1 ^c
AVP (ng/mL)	3.6 ± 0.5	1.5 ± 0.2 ^a	0.8 ± 0.03	0.9 ± 0.2	24.5 ± 1.8	5.4 ± 0.3 ^a	0.2 ± 0.01 ^{bc}
Hb (g/dL)	14.2 ± 0.1	19.2 ± 0.1 ^a	13.2 ± 0.1	18.2 ± 0.2 ^a	13.7 ± 0.2	16.5 ± 0.3 ^a	17.6 ± 0.3 ^{bd}
Hct (%)	39.7 ± 0.4	50.4 ± 0.5 ^a	38.8 ± 0.4	54.7 ± 0.7 ^a	42.5 ± 0.5	51.5 ± 0.7 ^a	NA
SpO ₂ (%)	98 ± 1.0	88.4 ± 0.2 ^a	97.4 ± 0.2	88.5 ± 0.5 ^a	96.9 ± 0.5	88.1 ± 0.4 ^a	87.6 ± 0.5
HR (beats/min)	64.1 ± 0.5	72.5 ± 1.5 ^a	63.6 ± 1.4	70.8 ± 1.5 ^a	61.9 ± 1.6	72.8 ± 1.9 ^a	67.7 ± 1.1 ^b

Values are expressed as mean ± SEM. Level of significance: $P < .05$.

AVP, arginine vasopressin; HA, high altitude; Hb, hemoglobin; Hct, hematocrit; SL, sea level; SpO₂, arterial oxygen saturation; HR, heart rate; NA, data not available.

SL vs HA (^a); Rajput vs HA native (^b); South Indian vs HA native (^c); Gorkha vs HA native (^d).

landers. Reduction in plasma proANP₁₋₉₈ at HA was 86% in Rajputs, 83% in Gorkhas, and 60% in South Indians compared with that at SL. Similar to SL findings, ethnicity ($P < .001$) but not age variation in plasma proANP₁₋₉₈ was observed among lowlanders. Also, plasma levels in South Indians were significantly ($P < .05$) high among lowlanders, and there was no variation in plasma levels between Rajputs and Gorkhas.

In HANs, plasma levels were within the SL normal range. Their values were intermediate between SL values of South Indians and those of North Indians (Rajputs and Gorkhas). proANP₁₋₉₈ levels in lowlanders at HA were significantly less than those in HANs. There was no age variation in HANs.

Discussion

To our knowledge, this study is the first to report on plasma proANP₁₋₉₈ level during prolonged HA exposure in relation to age and ethnicity with respect to the Indian population. The major findings of this study include decrease in proANP₁₋₉₈ levels in response to HA stress in lowlanders; variation in proANP₁₋₉₈ among different ethnic groups at HA; and absence of age-related variation in proANP₁₋₉₈ response to HA in lowlanders. In Ladakhis, who are HANs of the Himalayas, plasma proANP₁₋₉₈ level was within the SL normal range and did not exhibit age variation.

In lowlanders, plasma proANP₁₋₉₈ levels at SL were similar to the values reported in other populations using enzyme-linked immunosorbent assay (Biomedica Gruppe, Vienna, Austria).² Boomsma et al²⁰ have reported that a

Table 3. Plasma proANP₁₋₉₈ levels (nmol/L) in lowlanders and HA natives

Age group (years)	Lowlanders						HA natives (HA only)
	Rajput		Gorkha		South Indian		
	SL	HA	SL	HA	SL	HA	
20–24	0.65 ± 0.10 <i>n</i> = 10	0.12 ± 0.04 ^a	0.53 ± 0.07 <i>n</i> = 8	0.07 ± 0.02 ^a	1.84 ± 0.29 ^{ce} <i>n</i> = 10	0.75 ± 0.12 ^{acd}	0.76 ± 0.10 ^{ef} <i>n</i> = 11
25–29	0.75 ± 0.14 <i>n</i> = 11	0.19 ± 0.04 ^a	0.52 ± 0.06 <i>n</i> = 10	0.10 ± 0.03 ^a	1.18 ± 0.17 ^{bce} <i>n</i> = 12	0.48 ± 0.08 ^{acd}	1.15 ± 0.17 ^{efg} <i>n</i> = 10
30–34	0.72 ± 0.09 <i>n</i> = 12	0.10 ± 0.03 ^a	0.50 ± 0.08 <i>n</i> = 11	0.09 ± 0.02 ^a	1.63 ± 0.13 ^{ce} <i>n</i> = 9	0.58 ± 0.06 ^{acd}	1.16 ± 0.33 ^{efg} <i>n</i> = 7
35–50	0.67 ± 0.07 <i>n</i> = 15	0.12 ± 0.03 ^a	0.66 ± 0.11 <i>n</i> = 11	0.09 ± 0.02 ^a	1.57 ± 0.17 ^{ce} <i>n</i> = 12	0.77 ± 0.09 ^{acd}	1.33 ± 0.19 ^{efg} <i>n</i> = 12

Values are expressed as mean ± SEM. Level of significance: $P < .05$.

HA, high altitude; SL, sea level.

SL vs HA (^a); 20–24 years vs 25–29 years (^b); Rajput vs South Indian (^c); Gorkha vs South Indian (^d); Rajput vs HA native (^e); Gorkha vs HA native (^f); South Indian vs HA native (^g).

single blood sample, obtained after 30 minutes of quiet sitting as in our study, can give reliable and reproducible basal values. At SL, it can be observed that proANP₁₋₉₈ exhibits only ethnicity but not age variation. Such variation in physiologic concentrations of hormones involved in body fluid–electrolyte regulation in the Indian population has not been reported previously. In these individuals, BMI was within normal range, and there was no ethnic variation. Probably, the genetic expression of ANP differs among lowlanders. Although diet was similar in all the study subjects, it has been reported that dietary salt intake in urban southern India is higher than current recommendations for the general population.²¹ In the Framingham Study, it has been identified that 40% of the total variation in plasma natriuretic peptide levels is attributable to additive genetic effects in men.¹⁰

During HA exposure, changes in plasma proANP₁₋₉₈ in lowlanders was within the normal range. A uniform finding is that proANP₁₋₉₈ levels showed a decrease in all lowlanders irrespective of ethnicity. This is probably indicative of the fact that ANP secretion decreases after 3 to 4 weeks of stay at HA. It has been reported^{22,23} that after 4 weeks of stay at HA, plasma ANP level decreases due to altitude diuresis. At this point, it can be observed that plasma AVP was also reduced in all subjects of lowland origin. Reduction in AVP at HA is known to promote diuresis and is a characteristic feature of acclimatized subjects.²⁴ In addition, the rise in plasma protein and hematocrit in lowlanders at HA is indicative of plasma volume contraction. Thus, diuresis resulting in depletion of total body water might have inhibited ANP release, which is reflected by a parallel decrease in plasma proANP₁₋₉₈ levels. Also, the trend of resting heart rate remaining slightly above pre-ascent values and decrease in plasma volume are suggestive of ongoing acclimatization processes in lowlanders at HA. Several other factors could also have contributed to the decrease in plasma proANP₁₋₉₈ in lowlanders at HA. During chronic HA exposure, decrease in ANP levels despite a sustained increase in PAP has been attributed to downregulation of hypoxia inducible factor-1 α .⁷ Recently, it has been reported that plasma levels of N-terminal probrain natriuretic peptide, which is known to directly reflect right ventricular function and ANP, do not show any significant change despite an increase in pulmonary artery systolic pressure at HA.²⁵ Besides these factors, SpO₂ values above 85% in lowlanders at HA suggests that arterial hypoxemia was not severe enough to trigger ANP release.²⁶ Under HA stress, ethnicity but not age variation in plasma proANP₁₋₉₈ was observed among lowlanders. Although Rajputs and Gorkhas were studied in a relatively colder environment than that of South Indians at HA, it has been reported²⁷ that cold adaptability at HA was better only in Rajputs and Gorkhas than in South Indians. Also, it has been observed that ANP

was normal in soldiers who stayed above 6000 m for >10 weeks at -30°C .²⁸ Differences in humoral response between blacks and whites after plasma volume contraction has been observed.²⁹ A similar phenomenon could have contributed to the difference observed between North Indians (Rajputs and Gorkhas) and South Indians at HA. This aspect needs further research in relation to other fluid regulatory hormones at HA. Plasma proANP₁₋₉₈ level in lowlanders was less than that of HANs at HA. Ramirez et al³⁰ have reported that HANs excrete a salt load with increased facility than do SL natives exposed to HA, but the factors mediating these mechanisms were found to be independent of hypoxemia-induced alterations of aldosterone or ANP.

In this study on Ladakhis, who are HANs of the Himalayas, plasma proANP₁₋₉₈ levels were between the SL values of South Indians and North Indians (Rajputs and Gorkhas). This finding requires further research with respect to the Indian population. Antezana et al⁸ have observed that plasma ANP levels in HANs were less than those in SL natives despite high PAP. Plasma proANP₁₋₉₈ levels in HANs of this study are higher than the ANP values reported for Han Chinese living at 4300 m for 14 years. This difference is probably due to the fact that Han Chinese are relative newcomers to HA. Hence the high circulating proANP₁₋₉₈ levels in Ladakhis may reflect an adaptational change through generations. Age-related variation in plasma proANP₁₋₉₈ in Ladakhis between 20 to 50 years of age was absent. This is an important observation as it is well known that the incidence of chronic mountain sickness increases with age, and the disease is associated with overproduction of ANP.^{12,31,32} The hemoglobin value of <21.3 ³² in HANs of this study rules out excessive erythrocytosis.

In conclusion, HA stress is associated with significant reduction in plasma proANP₁₋₉₈ levels in lowlanders irrespective of age and ethnicity. This probably reflects a decrease in ANP release into the circulation during prolonged exposure to HA due to plasma volume contraction. Ethnicity but not age seems to influence proANP₁₋₉₈ levels at HA. In HANs, plasma proANP₁₋₉₈ was between the SL values of South Indians and North Indians (Rajputs and Gorkhas), did not exhibit age variation, and was higher than the plasma levels of lowlanders at HA. However, the results need to be interpreted with careful consideration of small sample size, lack of higher age groups, and lack of direct body-fluid and hemodynamic measurements.

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