THE EFFECT OF HYPOXIA ON BETA-ADRENORECEPTORS IN LYMPHOCYTES OF HIGHLANDERS.

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RESUMEN: El efecto de la hipoxia sobre los receptores beta-adrenérgicos en linfocitos de residentes de altura

Para evaluar el posible rol de la desensibilización de los receptores beta-adrenérgicos (β-AR) en la génesis de la hipertensión pulmonar de altura (HPAH), estudiamos residentes de altura en el Centro Nacional Kyrgyz de cardiología y medicina interna en Bishkek (760 m). Se demostró que estos sujetos sanos representaban un grupo heterogéneo de 3 subpoblaciones: 1) no respondedores; 2) hiper- respondedores; 3) con HPAH moderada. En estos tres subgrupos se determinó las diferencias de la respuesta de los β-AR a la hipoxia. En los no respondedores la B_{max} en normoxia estaba disminuida en comparación con los normo-respondedores y la hipoxia la incrementaba hasta el nivel de los no respondedores. En los residentes de altura con HPAH moderada la B_{max} estaba inicialmente alta e incrementó más en condiciones de hipoxia. Los estudios de activación de adenilato-ciclasa (cAMP) en condiciones hipóxicas en los linfocitos sanguíneos demostraron que el nivel de cAMP tanto basal como estimulado por agonista estaban reducidos en los tres grupos de residentes de altura, lo que revelaba un desacoplamiento de los β-AR con la proteína G y el desarrollo de desensibilización de los receptores.

Palabras claves: Hipertensión arterial pulmonar de altura; Residentes de altura; Linfocitos humanos; Receptores beta-adrenérgicos; Adenilato-ciclasa; Desensibilización.

RÉSUMÉEffet de l'hypoxie sur les récepteurs adrénergiques bêta des lymphocytes des habitants de haute montagne.

Pour évaluer le rôle possible de la désensibilisation des récepteurs adrénergiques bêta (ß-AR) dans la genèse de l'hypertension pulmonaire d'altitude (HPAH), nous avons réalisé une étude des habitants de haute montagne au Centre National Kyrgyz de cardiologie et de médecine interne de Bishkek (760 m d'altitude). Il a été démontré que ces sujets sains représentaient un groupe hétérogène de 3 sous-populations 1) non-répondants; 2) hyper-répondants; 3) avec HPAH modérée. Dans ces trois sous-groupes ont été déterminées les différences entre les réponses des ß-AR à l'hypoxie. Chez les non-répondants la B_{max} en normoxie était déprimée en comparaison avec les normo-répondants et l'hypoxie la faisait croître jusqu'au niveau des non-répondants. Chez les montagnards à HPAH modérée la B_{max} était initialement élevée et elle augmenta encore dans des conditions hypoxiques. Les études d'activation d'adénylate-cyclase (cAMP) dans des conditions hypoxiques ont démontré que dans les lymphocytes du sang le niveau de cAMP, aussi bien basal que stimulé par agonistes, était déprimé dans les trois groupes, révélant un désaccouplement des ß-AR et de la protéine G_s et le développement de la désensibilisation des récepteurs.

Mots-clés : Hypertension artérielle pulmonaire de haute altitude, Habitants des hautes montagnes, Lymphocytes humains, Récepteurs adrénergiques bêta, Adénylate-cyclase, Désensibilisation.

SUMMARY: In order to assess the possible role of ß-AR desensitization in the genesis of high-altitude pulmonary hypertension (HPAH), we conducted investigations of healthy highlanders in the Kyrgyz National Center of cardiology and internal medicine in Bishkek (760 m above sea level). It was shown that these conditionally healthy highlanders represented the heterogeneous group consisting of 3 subpopulations: 1) nonresponders; 2) hyperresponders; 3) with moderate HPAH. In these three groups the discrepancies of the ß-AR response to hypoxia were determined. In nonresponders hypoxia did not change the amount of ß-AR (B_{max}). In hyperresponders under normoxia BMax was decreased compared to normoresponders and hypoxia increased it up to the level of nonresponders. In highlanders with moderate HPAH BMax was initially high and it further increased under hypoxic conditions. Our studies of adenylate cyclase activation under hypoxic conditions demonstrated that basal and agoniststimulated cAMP level in blood lymphocytes were reduced in all three groups of highlanders, that revealed the uncoupling of , ß-AR with Gs-protein and the development of receptor desensitization.

Key Words : High altitude pulmonary arterial hypertension; Highlanders; Human lymphocytes; ß-adrenoreceptors; Adenylate cyclase; Desensitization.

INTRODUCTION

It is known that sensitivity to hypoxia in various animals and in humans is very variable. Concentration of norepinephrine in blood is increased when people rise at altitude 5400-6300 m above the sea level (1). It was demonstrated that in healthy lowlanders after 8 days of residence at 4350 m above sea level the blood norepinephrine level was increased and amount of ß-AR on lymphocytes decreased (2). 3-5 hours after returning to the lowland the receptor amount comes back to the initial level. Thus adrenoreceptors could play an essential role in adaptation to hypoxia. Moreover, hypernorepinephrinemia which associated with high-altitude hypoxia (3), results in desensitization of ß-AR. Infringements in the adrenoreceptor state, possibly, lead to disorders of organism adaptation to high altitude and to diseases, associated with the loss of adaptation to hypoxia. However, in the majority of investigations conducted in this field only the effect of hypoxia on adult lowlanders was studied.

At the same time, there are a lot of evidences that high altitude diseases, such as Monge's disease, high-altitude pulmonary arterial hypertension

(HPAH), chronic mountain sickness, affect mainly native residents of high altitude. People with hyperreactivity of pulmonary vessels to hypoxia are found more often among the residents of high altitude than among the lowlanders (4). Long-term studies of Kyrgyz Institute of cardiology have shown that development of severe HPAH with right ventricular hypertrophy (HRV) occurred more frequently among the highlanders than in the residents coming to high altitude at mature age. The same investigations demonstrated that, on the other hand, the most of native highlanders had higher stability to the development of high altitude sickness (5). For 7 years we have conducted our studies of ß-AR state in the highlanders native to Pamir and Tien-Shan, permanent residents of the altitudes of 3000-4200 M above the sea level.

We demonstrated that development of HAPH in highlanders correlated with desensitization of their ß-AR (6). In this study we investigate the correlation of the responses of ß-AR and pulmonary arterial blood pressure to hypoxia in healthy highlanders. Our study demonstrated that resistance of ß-AR to desensitization in hypoxia correlated with resistance of the organism to HAPH.

MATERIALS AND METHODS

Subjects studied

For investigation of ß-AR desensitization role in the genesis of HAPH with RVH of the heart, we studied healthy highlanders (without symptoms of RVH and HAPH, which were defined by the indirect methods) in Bishkek (760 m above the sea level). Mean pulmonary arterial pressure (PAP) was measured by the invasive method at the rest and after the inhalation of the hypoxic gas-mixture (10% 02). The thickness of the front wall of the right ventriculum was examined by the 2D-echocardiography.

According to PAP response to hypoxia these healthy highlanders were divided into three groups:

1. nonresponders
2. hyperresponders
3. hyperresponders with moderate HPAH.

Blood samples were collected before and after hypoxia and the state of ß-AR in the lymphocytes of peripheral blood was analyzed. According to the data of Brodde et al. (7) the state of ß-AR on lymphocytes reflected the state of ß-AR in right atrium of human heart.

Preparations of cells

Blood samples were drawn from highlanders into plastic tubes containing heparin. Lymphocytes were separated from whole blood samples according to method of Boum et al. (8). Blood samples were diluted with phosphate buffered saline (PBS) to twice the volume and layered over FicollVeragrafin (specifIc gravity 1,077). The tubes were centrifuged at 425g for 30 min. The layer of the lymphocytes was harvested and washed twice with PBS and once with 20 mM HEPES in MI99 (DMEM) containing 1 mg/ml BSA. The final cell suspension was diluted in DMEM. The vialbility of the cells were tested by excluding of trypan blue.

Radioligand binding

ß-AR were quantitated using ligand [^{3}H]-dihydroalprenplol ([^{3}H] DHA). The assays were performed in polypropylene test tubes. Incubations were performed in a total volume of 0,5 ml of DMEH containing 3-5x10^{6} cells. Non-specific binding was determined by performing incubations in the presence of 1 mM propranolol. The reactions were terminated by adding three volumes of ice-cold 10 mM Tris buffer and samples were filtered (GF-C filters, Whatman). The receptor-bound ligand radioactivity on the filters was counted in liquid scintillation counter.

Adenylate cyclase activity

ß-Adrenergic-stimulated adenylate cyclase activity was assayed by method of Solomon and reflected the rate of generation of [^{32}P] cAMP from alpha[^{32}P] ATP (9). Lymphocytes were incubated in balanced salt solution containing 1 mM isobutyl-methyl-xanthine (IBMX). The mixture was incubated for 10 min at 30°C. Incubations were terminated by addition of 0,2 ml 0,1 N HC1. A radioactivity of the samples was measured by method of Cherenkov.

Materials

[^{3}H] - dihydroalprenolol was purchased from Amersham (England), and Ficoll 400 from Pharmacia (Sweden). All other compounds were obtained from Sigma Chemical Co. (USA).

RESULTS.

According to PAP response to hypoxia we isolated 3 subpopulations of healthy highlanders: 1) nonresponders, 2) hyperresponders, 3) hyperresponders with moderate HPAH. Nonresponders had low PAP at the rest (12,8 ±1,6 mmHg), which moderately increased under hypoxia (21,6±2,9 mmHg). Hyperresponders also had low PAP at the rest (6,7±1,9 mm Hg), which increases after hypoxia (34,8±2,7 mm Hg). Highlanders with moderate HPAH had PAP 20,7±1,1 mmHg, which sharply increased, when we gave the hypoxia (Table 1).

The differences between groups of highlanders in ß-AR response to hypoxia were examined. The ß-receptor density on lymphocytes of nonresponders in normoxia and under hypoxia was not significantly different (Fig. 1). In hyperresponders Bmax was much lower in normoxic conditions but under hypoxia increased to the level of nonresponders (3,7±1,4 and 6,9±1,8 fmol/10^6 cells, respectively). In highlanders with moderate HPAH BMax was initially high and it was further increased under hypoxia (9,5±1,4 and 12,1 ± 1,0 fmol/ 10^6 cells).

After 5 years we investigated , ß-AR density in lymphocytes and their response to hypoxia in the same subjects (3 nonresponders and 6 hyperresponders). We demonstrated that Bmax did not change both in hyperresponders and nonresponders (4.0±1.8 and 7.5±2.7 fmol/ 10^6 cells). When we incubated these lymphocytes in hypoxic conditions (hypoxia in vitro) for 48 hours we found that lymphocytes of nonresponders did not change their density (6.9±1.3 fmol/10^6 cells), where as lymphocytes of hyperresponders dramatically changed Bmax (4.0±1.8 to 7.7±1.1 fmol/10^6 cells).

Table 1. Parameters of the small circle haemodynamic, ß-AR and AC activity in highlanders

Group	Test	TWR V	PAP	B_{MAX}	Basal	AC activity	
			(mmHg)		activity	Iso	Forsk
Non	N	36±2	12,8±1,6	6,8±2,3	7,9±0,8	10,4±1,1**	10,2±1,3**
	H		21,6±2,9*	7,2±1,9	7,1±0,8	11,2±1,6**	14,1±1,6**
Hyper	N	36±3	16,7±1,9	3,7±1,4	7,4±0,4	10,2±1,3**	12,1±1,3**
	H		34,8±2,7*	6,9±1,8***	7,5±1,1	10,1±1,8**	14,3±1,1**
With Mode-	N	36±3	20,7±1,1	9,5±1,4	20,2±1,9	23,5±1,5**	27,5±2,1**
rateHPAH	H		36,2±2,9*	12,1±1,0***	23,2±1,5	23,2±2,5**	26,4±3,0**

*- $p < 0,0001$ normoxia against hypoxia; ** -$p < 0,001$ basal activity against stimulated; *** - $p < 0,005$ B^{max} under normoxia against hypoxia.

Concentration of B_{max} was measured in fmol/10^6cells,Iso and Forsk 10^{-5}-10^{-6}M.

N - normoxia; H - hypoxia

The activation of adenylate cyclase (AC)

AC basal activity was not significantly different in both non- and hyperresponders before and after hypoxic test (Table 1). Isoproterenol stimulation in normoxic conditions led to almost identical increase of AC activity in both groups, but under hypoxia only in nonresponders the activation was slightly increased, whereas in hyperresponders percentage of activation did not change authentically.

Stimulation of AC was similar in hyper- and nonresponders . We have demonstrated above that B_{max} was two times lower in hyperresponders compared to nonresponders, so these data revealed hyperreactivity of ß-AR in hyperresponders. Hypoxia did not change isoproterenol- stimulated activation of AC . In highlanders with moderate HPAH basal activity of AC was significantly higher compared to non-and hyperresponders. Hypoxia blocked effect of isoproterenol and forskolin on AC. These data revealed desensitization of ß-AR with development of HPAH.

Our measurements of cAMP level in lymphocytes after hypoxia in vitro demonstrated that hypoxia decreased the basal and agonist-stimulated cAMP levels in hyper- and normoresponders (Table 2).

Figure 1

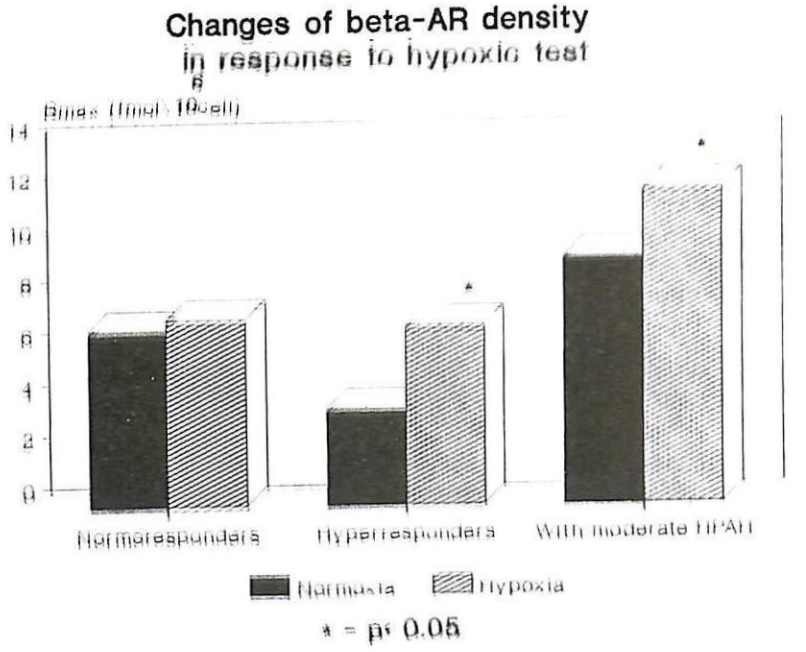

Table 2. CAMP concentration (pmol/in ml cells in highlanders

Group	Test	Basal activity	AC activity	
			Iso 10^{-5}M	Forsk 10^{-4}M
Normoresponders	Norm	7,2±0,8	8,1±0,4**	8,4±0,3**
	Hyp	3,2±0,2	3,1±1,0,1***	8,4±0,4**
Hyperresponders	Norm	5,7±0,3	6,3±0,3**	6,6±0,2**
	Hyp	3,0±0,06	3,6±0,04***	5,7±0,4**

Where *- the discrepancies between control and hypoxia are authentic with $p<0,05$; ** - $p<0,05$ basal activity against stimulated; *** - non-significant.

DISCUSSION

It is known that at mammals hypoxia increased blood norepinephrine levels and it could result in desensitization of ß-AR (1). At the same time, preservation of normal sympathetic reactivity is the important factor of the individual survival in extremal environment. Our data demonstrated the heterogenic responses of human organism to hypoxia and would allow us to forecast the clinical prognosis of chronic hypoxia on developing HPAH. It is known that ß-AR will stimulate vasorelaxation of the systemic and pulmonary vessels and decrease arterial blood pressure. Thus the functional disability of ß-AR will correlate with decreased vasorelaxation and increase in arterial blood pressure.

By dividing highlanders into three subgroups, we found out the correlations between the ß-AR and PAP responses to hypoxia. In nonresponders hypoxia moderately increased PAP and did not change ß-AR density. In hyperresponders hypoxia increased ß-AR density and PAP. In highlanders with moderate HPAH Bmax was initially higher and hypoxia increased it furthermore. When we investigated the functional activity of ß-AR by isoproterenol dependent activation of AC we found that hypoxia did not change the rate of activation of AC both in hyper- and normoresponders. However, in hyperresponders under hypoxia it was almost 2-fold increase of ß-AR amount which was not accompanied by proportional increase in AC activity. These data proved that hypoxia recruited part of ß-AR which were still not coupled with Gs protein and AC (10). In nonresponders hypoxia did not affect both ß-AR amount and their activity. In highlanders with moderate HPAH isoproterenol moderately activated AC in normoxia and did not stimulate it in hypoxia. Thus, in this group the ß-AR desensitization becomes obvious already in normoxia and hypoxia enhances it furthermore.

High basal activity and disappearance of

isoproterenol dependent activation of AC revealed that AC activity in this group of subjects was uncoupled from ß-AR and had have different mechanisms of regulation (11). It is known that elevation of intracellular calcium, activation of PKC could activate AC by ß-AR independent pathways (12).

Interestingly, that lymphocytes from hyperresponders could upregulate ß-AR density in response to hypoxia not only in vivo but in vitro. Nonresponder's lymphocytes did not change Bmax to hypoxia in vitro. Early we had shown that hypoxia upregulated different proteins in lymphocytes and this effect was mediated by calcium (13). When added calcium scavenger EGTA ablated the effect of hypoxia on protein expression (14). We demonstrated that hyperresponders and highlanders with moderate HPAH exhibited hyperactivity of their calcium channels and increased intracellular calcium levels (15). We suggest that increased intracellular calcium might upregulate expression of ß-AR and hyperactivation of AC in highlanders with moderate HPAH.

Our data suggest that healthy highlanders are heterogeneous by their response to hypoxia. The difference in response of pulmonary arterial blood pressure to hypoxia is correlated with the response of ß-AR to hypoxia. In hyperresponders hypoxia upregulates ß-AR density which does not enhance signal transduction because of partial desensitization of ß-AR. In the group with moderate HPAH most of ß-AR are desensitized and AC activity is not regulated by AR. Thus, the resistance to development of HPAH is correlated with the resistance of ß-AR to desensitization in hypoxia, and its ability to setivate AC and conduct vasodilatation of pulmonary arteries.

CONCLUSIONS

1. According to the response of pulmonary arterial pressure and ß-adrenoceptors to hypoxia, healthy highlanders appeared to represent three subgroups: nonresponders, hyperresponders, highlanders with moderate HPAH.
2. Hyperresponders and highlanders with moderate HAPH have signs of desensitization of ß-AR which correlates with hyperresponse of pulmonary arterial blood pressure to hypoxia. Resistance of highlanders to the development of HPAH is correlated with the resistance of their ß-AR to hypoxia-induced desensitization.

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