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ORIGINAL ARTICLE

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Effects of prolonged high-altitude exposure on peripheral adrenergic receptors in young healthy volunteers

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Abstract The regulation of adrenergic receptors during hypoxia is complex, and the results of published reports have not been consistent. In the present study blood cell adrenoceptor characteristics at sea level (SL) before and after prolonged exposure to high altitude (HA) were measured in seven trained young male lowlanders. Sympathoadrenal activity and clinical haemodynamic parameters were also evaluated before departure (SLB), after 1 week (HA1) and 4 weeks (HA4) at HA and 1 week after return to sea level (SLA). As compared to pre-departure sea level values, urinary norepinephrine excretion increased significantly during altitude exposure [SLB: 10.26 (3.04) μg · 3 h^{-1}; HA1: 23.2 (4.19) μg · 3 h^{-1}; HA4: 20.3 (8.68) μg · 3 h^{-1}] and fell to pre-ascent values 1 week after return to sea level [SLA: 9 (2.91) μg · 3 h^{-1}]. In contrast, mean urinary epinephrine levels did not increase over time at HA. Both systolic and diastolic blood pressures, as well as heart rate, were increased during HA exposure. The circadian blood pressure and heart rate rhythms were preserved during all phases of altitude exposure. Mean maximal binding (B_{max}) of the α_2-adrenoceptor antagonist [^{3}H]rauwolscine to platelet membranes was significantly reduced (P < 0.001) after exposure to chronic hypoxia [SLB: 172.6 (48.5) fmol · mg^{-1} protein versus SLA: 136.8 (56.1) fmol · mg^{-1} protein] without change in the dissociation constant (K_D). Neither the lymphomonocyte β_2-adrenoceptor B_{max} [SLB: 38.5 (13.6) fmol · mg^{-1} protein, versus SLA: 32.4 (12.1) fmol · mg^{-1} protein] nor the K_D for [^{3}H]dihydroalprenolol was affected by chronic hypoxia. Cyclic AMP (adenosine 3′,5′-cyclic monophoshate) generation in lymphomonocytes by maximal isoproterenol stimulation was not modified after prolonged HA exposure. In conclusion, the down-regulation of α_2-adrenoceptors appears to be an important component of the adrenergic system response to HA exposure.

Key words Adrenergic receptors · Epinephrine · High altitude · Hypoxia · Norepinephrine

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Introduction

High altitude (HA) exposure above 3000 m is associated with several modifications of physiological parameters that are modulated by endocrinological and neurological factors (Blume 1984). During prolonged HA exposure, activation of the sympathoadrenal system has been reported both in humans (Rostrup 1998) and in animals (Johnson et al. 1983). Plasma and urine catecholamine measurements demonstrate that HA exposure leads to an initial and brief increase of epinephrine, followed by a progressive increase in the sympathetic neurotransmitter norepinephrine (Johnson et al. 1988). This hyperadrenergic condition is significant in that the adrenergic and noradrenergic systems play a crucial role in the cardiovascular and metabolic adaptation associated with HA exposure (Mazzeo et al. 1991; Savourey et al. 1998).

Since adrenergic receptors are important sites of regulation of cardiac function and vascular tone, several studies of the effects of hypoxia on adrenergic receptors have been undertaken in animals and in humans. In rats, chronic HA exposure is associated with either no change (Winter et al. 1986) or a decline (Mader et al. 1991) in the density of left ventricular β-adrenoceptors. Differences in the modulation of β-adrenoreceptor number in response to chronic hypoxia have been related to the age of the animals (Kacimi et al. 1992) and to the duration of exposure to hypoxia (Voelkel et al. 1981).

By necessity, human studies have been performed using blood cells, particularly lymphocytes and granulocytes for β_2-adrenoceptors and platelets for α_2-adrenoceptors. Conflicting results have been reported regarding β_2-adrenoceptor density. Antezana et al. (1994) observed a reduction of β_2-adrenoceptor density with no change in affinity in a population of lowlanders exposed to extreme HA, while Zaccaria et al. (1997) reported that the number of β_2-adrenoceptors was not changed after prolonged exposure to similar climatic conditions.

The effect of HA exposure on α_2-adrenoceptor binding sites has been studied less extensively than it has on β-adrenergic receptors. Recently, Zaccaria et al. (1997) demonstrated a decrease in platelet α_2-adrenoceptor number and affinity after prolonged exposure to altitude hypoxia.

Therefore, we see that studies on the effects of hypoxia on β_2-adrenergic receptor density have divergent results and that the few data on regulation of α_2-adrenoceptors at HA exposure should be confirmed or refuted by further studies. Thus, we have re-examined the modifications occurring in both lymphomonocytic β_2-adrenoceptor density, affinity and function, and in platelet α_2-adrenoceptor density and affinity in a group of trained healthy male lowlanders before and after 5 weeks of HA exposure. Sympathoadrenal activity and clinical haemodynamic parameters were also measured in these subjects. Our results were compared with those of the most relevant studies found in the literature in this field.

Methods

Subjects

Seven healthy young male volunteers [age 24.5 (0.6) years] were recruited for the study. They were expert, trained climbers who participated in a scientific Himalayan expedition to the "Piramide" laboratory station (5050 m) in Kumbu valley (Nepal), requiring prolonged (5 weeks) HA exposure. All the subjects were lowlanders and had not been exposed to HA within the preceding 6 months. They did not assume any pharmacological therapy. All the subjects gave informed consent, and the institutional committee on human research approved the study protocol. The research was part of the Italian High Altitude Project.

Study protocol

The study was divided into four phases: sea level before departure (SLB), 1 week (HA1) and 4 weeks (HA4) after arrival at HA (5000 m), and 1 week after return to sea level (SLA). During the entire period of residence at HA a high caloric diet was maintained with the percentage distribution of fat, carbohydrates and proteins equal to about 30%, 55% and 15%, respectively. Fluid intake at HA was recommended to be greater than 2 l/day in addition to fluid foods. The daily level of physical activity of about 3 h walking was also comparable for all the participants in the study. During the 3rd week of stay at HA the subjects were programmed for a climbing excursion to 7000 m. In each phase of the study the subjects underwent 24-h blood pressure and heart rate monitoring via a non-invasive automatic cuff apparatus (Spacelabs) and a 3-h urine collection period for determination of catecholamine levels. During the collection period they were seated in a quiet room at similar temperature conditions in each phase of the study. Before departure and after return to sea level two blood samples were drawn from the antecubital vein after the subject had rested in a supine position for 30 min. The samples were then prepared for isolation of platelets and lymphomonocytes to perform the adrenoceptor study.

Laboratory measures

Isolation of platelets and lymphomonocytes

Platelet-rich plasma (PRP) was obtained from the blood sample anticoagulated with 3.2% sodium citrate solution, by centrifugation at 200*g* for 10 min (4 °C) as described previously (Carretta et al. 1992). Upon removal of plasma, an equal volume of isotonic solution was mixed with the blood and centrifuged at 200*g* for 10 min. The supernatant was removed and added to the PRP collected previously. The PRP was centrifuged at 17,000*g* for 15 min at 4 °C. The platelet pellets were washed twice with 8 ml of an isotonic buffer solution, pH 7.35, containing Tris-HCl 50 mM, NaCl 120 mM, EDTA 20 mM and again centrifuged at 17,000*g* for 15 min at 4 °C. After the second wash the pellets were resuspended in ice-cold hypotonic buffer (Tris-HCl 5 mM, EDTA 5 mM, pH 7.5) and centrifuged at 30,000*g* for 15 min at 4 °C. The platelet pellets were resuspended in a single tube, washed with ice-cold incubation buffer (Tris HCl 120 mM, EDTA 0.5 mM, pH 7.4) and centrifuged again at 30,000*g* for 15 min at 4 °C. The final pellet was resuspended in ice-cold incubation buffer to provide a protein concentration of 1 mg $\cdot$ ml^{-1} and immediately used for the binding assay.

Lymphomonocytes were isolated from the blood sample anticoagulated with heparin according to the method of Boyum (1968). Fresh heparinized whole blood was diluted 1:1 with isotonic saline solution. Diluted blood was applied to the top of a 3-ml Ficoll-Hypaque mixture. After centrifugation at 500*g* for 15 min the plasma layer was removed and the lymphomonocyte band was harvested by vacuum aspiration and divided into two aliquots. Each of the two aliquots was washed twice in hypotonic saline solution to haemolyse the remaining red cells. The mononuclear cells isolated were 93–98% lymphomonocytes. After the final wash one of the two lymphomonocyte preparations was resuspended in Hanks' balanced salts (HBSS) for cAMP determination. The remaining lymphomonocyte pellet was resuspended in incubation buffer (Tris HCl 50 mM, $MgCl_2$ 10 mM, pH 8), and the suspension was lysed by Polytron action for 12 s. Incubation buffer was used to wash the suspension into a chilled Sorvall tube that was centrifuged at 4 °C (1900*g*) for 10 min. The pellet was resuspended in 3–5 ml incubation buffer for a final concentration of 1–1.5 mg protein $\cdot$ ml^{-1} and stored at −70 °C for later binding assay.

The protein content of the platelet and lymphomonocyte membranes was assessed according to the Lowry method (Lowry et al. 1951).

α_2-Adrenoceptor binding assay

Aliquots of platelet suspensions (150 μl) were incubated with different concentrations (from 0.25 to 10 nM) of [^{3}H]rauwolscine (Amersham, Italy) to obtain a final volume of 250 μl. The samples were then incubated at 22 °C for 30 min. Incubation was stopped by addition of ice-cold assay buffer and subsequent rapid vacuum filtration through Whatman GF/C filters. Radioactivity of the filters was then counted in a β-liquid scintillation analyser (Tri-Carb, Canberra Packard, Milan, Italy). Parallel incubation in the presence of phentolamine (10^{-5} M) was used to determine non-specific binding. Specific binding was defined as the difference between the total and non-specific binding. Non-specific binding was about 20% at 2 nM [^{3}H]rauwolscine.

β_2-Adrenoceptor binding assay

Lymphomonocytes were defrosted, centrifuged at 200*g* at 4 °C for 15 min and resuspended in incubation buffer (see above). The total number of β-adrenoceptors was measured using the lipophilic radioligand [^{3}H]dihydroalprenolol (Amersham, Italy). Saturation binding studies were performed by incubating aliquots of the membrane suspension (150 µl) with at least five concentrations of [^{3}H]dihydroalprenolol (ranging from 0.5 to 6 nM). Solutions of agonist were prepared in buffer containing ascorbic acid (1 mM) to reduce non-specific binding. Non-specific binding was determined by incubating the samples in the presence of 10^{-6} M (−)propanolol and was 20–25% of total binding. Incubations were carried out for 20 min at 37 °C and were terminated by diluting the incubation mix with 3 ml of ice-cold incubation buffer followed by rapid vacuum filtration through Whatman GF/C filters. The filters were washed four times with 3 ml of ice-cold incubation buffer, dried and counted in a β-liquid scintillation counter for determination of total binding. Specific binding was determined by subtraction of non-specific from total binding.

Adenylate cyclase assay

β-Adrenergic-mediated adenylate cyclase activity was determined by cAMP generation before and after stimulation with isoproterenol. The cell suspension (see above) was diluted to give a final concentration of $2 \cdot 10^6$ to $5 \cdot 10^6$ cells $\cdot$ ml^{-1}. The incubations were performed in a final volume of 1.9 ml including cell suspension (1 ml), theophylline (10 mM) and the presence of isoproterenol bitartrate (10^{-6} M) or ascorbic acid (blank) at 37 °C in an atmosphere of 95% O_2/5% CO_2 for 12 min. The incubation was terminated by centrifugation at 1500*g* for 3 min, and the supernatant was discarded. The precipitate was lysed by centrifugation with two 1-ml aliquots of ethanol/0.1 M HCl_2 (80:20) and the supernatants collected and evaporated to dryness. The residue was stored at −20 °C. The amount of cAMP was estimated after reconstitution of the sample in 1 ml distilled water by a radioimmunoassay method.

Catecholamines

Urinary catecholamines were measured by high-performance liquid chromatography (HPLC) with electrochemical detection.

Statistical analysis

Paired Student's *t*-tests were used to compare data before and after HA exposure. Parameters at different time points were compared by ANOVA for repeated measures. Linear regression analysis was used to test the two-variable relationship. Statview software by SAS Institute for the Apple Macintosh computer was used. Saturation binding data were analysed by the iterative curve-fitting program Ligand. Data are reported as the mean (SD). A value of $P < 0.05$ was considered statistically significant.

Results

Catecholamines

Urinary norepinephrine excretion was significantly increased ($P < 0.05$) as compared to pre-ascent values after 1 week of residence at 5000 m [SLB: 10.26 (3.04) µg $\cdot$ 3 h^{-1} versus HA1: 23.2 (4.19) µg $\cdot$ 3 h^{-1}]. Thereafter, excretion rates remained stable at this elevated level for the duration of the altitude exposure [HA4: 20.3 (8.68) µg $\cdot$ 3 h^{-1}]. One week after return to sea level the urinary norepinephrine excretion returned to the baseline values [SLA: 9 (2.91) µg $\cdot$ 3 h^{-1}] (Fig. 1). In contrast, the epinephrine excretion rates were similar to baseline values both after 1 and 4 weeks of residence at HA. After return to sea level a significant reduction in epinephrine excretion was observed (Fig. 1).

Cardiovascular measurements

Mean values of systolic and diastolic blood pressure and of heart rate are shown in Table 1. All the study participants were normotensive. Diurnal and nocturnal systolic and diastolic blood pressure recorded after 1 and 4 weeks at altitude were significantly increased compared to sea level values. The greatest percentage increase was observed in the diastolic values. Nevertheless, the circadian rhythm, with lower systolic and diastolic blood pressure at night, was preserved during all phases of the study. After return to sea level blood pressure values were similar to those recorded before departure.

Resting mean diurnal heart rate was significantly increased after 1 and 4 weeks at altitude. The nocturnal

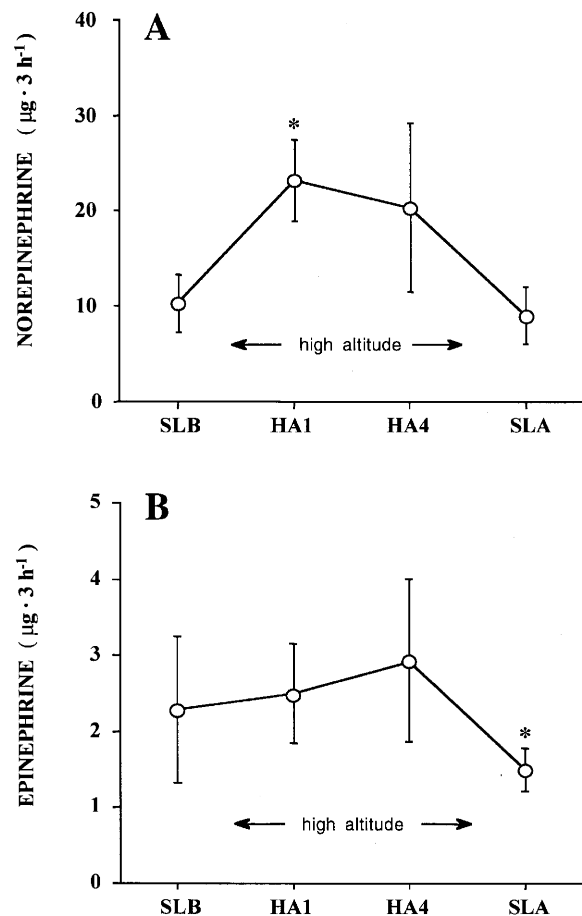

Fig. 1 Mean 3-h urinary norepinephrine (**A**) and epinephrine (**B**) concentrations at sea level before departure (*SLB*), after 1 (*HA1*) and 4 (*HA4*) weeks at 5000 m and 1 week after return to sea level (*SLA*). Values are expressed as mean (SD). *$P < 0.05$

Table 1 Twenty-four-hour mean ambulatory blood pressure and heart rate measurements. Measurements were made at sea level before departure (*SLB*), after 1 (*HA1*) and 4 weeks (*HA4*) of stay at high altitude and after return (*SLA*) from high altitude exposure. Values are expressed as mean (SD)

		Altitude exposure							
		SLB		HA1		HA4		SLA	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
SBP (mmHg)	Day	124	17*	144	13*,†	140	8**,†	125	5*
	Night	117	19	132	16†	125	11†	117	10
DBP (mmHg)	Day	66	8**	83	7††	80	6††,*	67	1**
	Night	57	9	76	10†	74	4††	62	3
HR (beats · min^{-1})	Day	65	10**	73	10**,†	87	13**,††	76	11**,††
	Night	50	8	49	5	58	9†	59	11†

Significantly different from night values ($P < 0.05$; **$P < 0.005$); †significantly different from sea level values before departure (†$P < 0.05$; ††$P < 0.005$)

reduction of cardiac activity was maintained during all phases of the study. Heart rate was also persistently elevated 1 week after return to sea level.

Adrenergic receptors

The mean maximal binding (B_{max}) of the platelet α_2-adrenoceptors evaluated 1 week after return to sea level was significantly reduced ($P < 0.001$) as compared to pre-ascent values [SLB: 172.6 (48.5) fmol · mg^{-1} protein versus SLA: 136.8 (56.1) fmol · mg^{-1} protein] (Fig. 2). The dissociation constant (K_D) for [^{3}H]rauwolscine, however, was not influenced by prolonged hypoxia exposure [SLB: 1.65 (0.23) nM versus SLA: 1.53 (0.32) nM] (Fig. 2). No variations in the lymphomonocyte β_2-adrenoceptor B_{max} [SLB: 38.5 (13.61) fmol · mg^{-1} protein versus SLA: 32.37 (12.03) fmol · mg^{-1} protein] or the K_D for [^{3}H]dihydroalprenolol [SLB: 1.21 (0.66) nM versus SLA: 1.1 (0.41) nM] were found after exposure to HA (Fig. 2). No correlation was observed between urinary catecholamines and the number of platelet α_2-adrenoceptors or the number of lymphomonocyte β_2-adrenoceptors.

cAMP accumulation

Mean intracellular levels of cAMP evaluated in lymphomonocytes in vitro, both at basal conditions and after stimulation with isoproterenol, before and after HA exposure are shown in Fig. 3. The isoproterenol-induced increase of cAMP evaluated 1 week after return to sea level was not modified as compared to the pre-ascent value. No correlation was observed between urinary catecholamines and the cAMP increase in lymphomonocytes.

Discussion

Altitude exposure significantly activated the sympathoadrenal system. Diurnal and nocturnal systolic and diastolic blood pressure increased at 5000 m during the study period. The heart rate showed a parallel shift to higher values during the 5-week stay at HA. Our data on the pattern of sympathoadrenal activity are consistent with previous findings (Antezana et al. 1994; Johnson et al. 1988; Mazzeo et al. 1998; Rostrup 1998). However, any comparison of studies on extreme altitude exposure must take into account that altitude hypoxia is not the only factor influencing sympathetic activity. During field studies, the increased sympathoadrenal

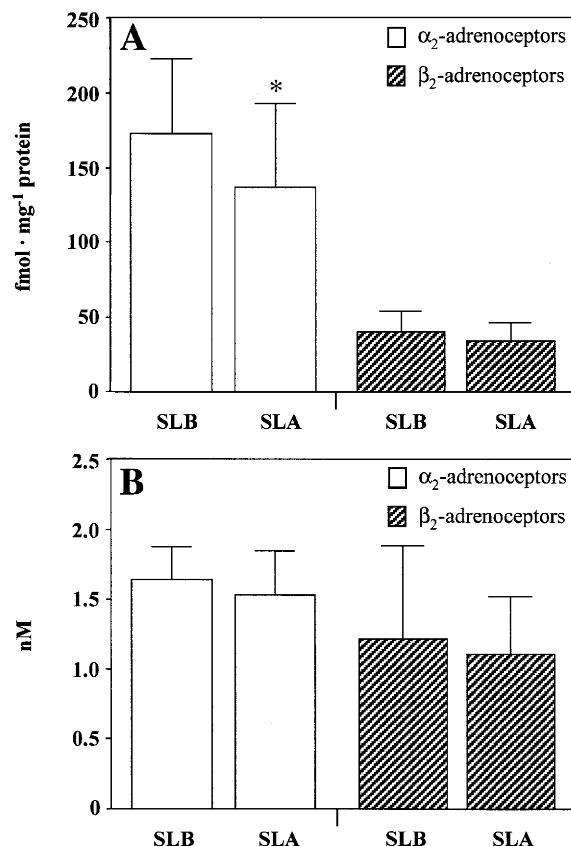

Fig. 2 Platelet α_2-adrenergic receptor (*white columns*) and lymphomonocyte β_2-adrenergic receptor (*shaded columns*) density (**A**) and affinity (**B**) before departure (*SLB*) and after return from exposure to high altitude (*SLA*). Values are expressed as mean (SD). *$P < 0.001$

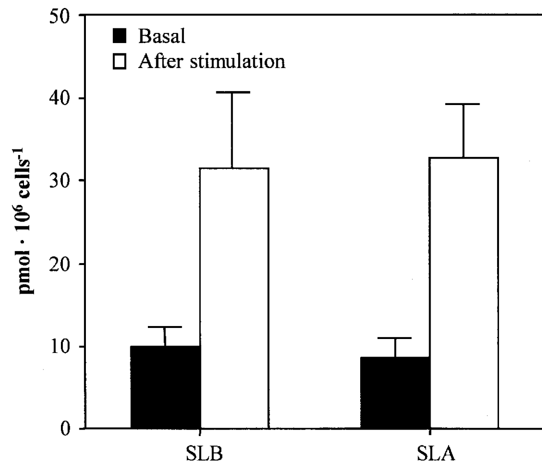

Fig. 3 Basal and β-adrenoceptor-stimulated cAMP production before (*SLB*) and after return from HA exposure (*SLA*). Values are expressed as mean (SD)

activity may be the result of the combined effects of altitude, cold temperature, wind, physical exercise, hypoglycaemia, dehydration and other less quantifiable factors such as psychological stress due to the exposure to a hostile and foreign environment (Rostrup 1998). Moreover, duration of stay, altitude and the subjects' residence of origin vary considerably between studies.

In our study the urinary excretion of norepinephrine and of epinephrine were chosen as markers of sympathetic and adrenal activation, respectively (Wolfel et al. 1994). While norepinephrine excretion increased after prolonged exposure to HA, epinephrine was not changed. Thus, sympathetic neural activity was predominant over the adrenal medullary response, and consequently norepinephrine secretion appeared to be the main factor responsible for chronic acclimatization to altitude.

In the present study systolic and diastolic blood pressures were consistently elevated during HA exposure and returned to baseline values after return to sea level. Although the present data cannot establish a causal relationship between the norepinephrine increase and the increase in blood pressure, the similar time course of such changes suggests, as previously reported (Wolfel et al. 1994), that the elevation in systemic arterial pressure relates to increased sympathetic activity from norepinephrine. However, the increased release of other hormonal factors such as vasopressin (Brahmachari et al. 1973; Rostrup 1998), cortisol (Sawhney et al. 1991) and thyroid hormones (Mordes et al. 1983) may also influence blood pressure control at HA. The concept that heightened sympathetic activity is responsible for the HA-related tachycardia is supported by this study. The persistence of a higher heart rate in the absence of increased urinary catecholamine excretion 1 week after return to sea level is in agreement with the nature of sympathetic nervous system responses, which are typically organ specific. It is possible that increased activity of the sympathetic system could affect heart rate independently of the increase of global indexes of sympathetic nervous system function, such as urinary catecholamines. However, activation of the sympathetic limb of the autonomic nervous system may not be the only factor involved in heart rate control at altitude. The extent to which altered parasympathetic activity or the presence of stable haemodynamic modifications such as the reduction of stroke volume (Vogel et al. 1974) contribute to the heart rate responses observed over time at altitude remains to be determined.

The main objective of this study was to evaluate the regulation of adrenergic receptors after prolonged exposure to HA. Binding studies performed with the α_2-selective radioligand [^{3}H]rauwolscine and intact platelets showed a significant reduction of the number of α_2 receptors without modification of receptor affinity 1 week after return to sea level as compared to the pre-expedition value. Our data on α_2-adrenoceptor density and affinity confirm previous observations by Ponchia et al. (1994) and Zaccaria et al. (1997), who studied the number of platelet-binding sites using [^{3}H]yohimbine and [^{3}H]UK 14,304 as ligands, respectively.

In the present study platelet α_2-adrenoceptors were maintained in a down-regulated state 1 week after return to sea level although urinary norepinephrine excretion had returned to the pre-ascent values. A likely explanation is that the number of platelet α_2-adrenoceptors is mainly regulated in parallel with sympathetic nerve activity rather than by circulating catecholamines (Noshiro et al. 1990). Activation of sympathetic outflow, at least in some organs, could continue once returned to sea level, as indirectly supported by the persistence of an increased heart rate. Since the mechanism of down-regulation of the α_2-adrenoceptors involves an increased rate of receptor degradation (Heck and Bylund 1997), another explanation for the delay in α_2-adrenoceptor repopulation on the platelet surface is that the absence of a nucleus precludes the synthesis of new receptors. Hence, the effect of altitude hypoxia on platelet α_2-adrenoceptors appears to be related to the mean life span of platelets which ranges between 6.9 and 9.9 days (Corash et al. 1978; Wessels et al. 1987).

The consequences of α_2-adrenoceptor down-regulation during altitude hypoxia are not known. Considering that human platelets respond to α-adrenergic stimulation by altering their aggregating properties, one might expect that the decrease in binding site number could be related to a decrease in platelet aggregating function. However, recent findings have demonstrated that bleeding time and the ability of platelets to aggregate are not altered at HA (Le Roux et al. 1992).

A relationship between α_2-adrenoceptor modifications and the haemodynamic adaptation to HA cannot be established by this study. Nevertheless, modifications of the α_2-adrenoceptors on the platelet surface may represent comparable changes at the cerebral level (Piletz et al. 1991). In the central nervous system α_2-adrenoceptors are known to play an important role in cardiovascular regulation (Dashwood et al. 1985), and the stimulation of these receptors in the ventrolateral

medulla has been shown to reduce sympathetic and to increase parasympathetic outflow. Although considerable caution should be exercised in the interpretation and extrapolation of these results from platelets to other tissues, the observed α_2-receptor down-regulation may lead to the well-known imbalance between sympathetic and parasympathetic activity which occurs during altitude exposure. This hypothesis could explain the delayed recovery of heart rate observed in our subjects after return to sea level.

In the present study lymphomonocytic β_2-adrenoceptor number and affinity were not modified after HA exposure. In the same subjects neither basal nor maximal enzymatic activity of adenylate cyclase evaluated after isoproterenol stimulation was affected by altitude hypoxia, suggesting that the mechanisms of coupling between the stimulatory guanine nucleotide binding protein and β-receptor seemed to be preserved. Our observations are in agreement with results obtained by Zaccaria et al. (1997), who used granulocytes as the source of β-adrenergic receptors. In contrast with our results, Antezana et al. (1994) found a significant decrease of lymphocyte β_2-adrenoceptor density with no change in affinity in a group of lowlanders during HA exposure. Several possible explanations may account for these conflicting results. Influences of physical activity on the β-adrenoceptor system have been widely described in the literature (Eysmann et al. 1996; Krawietz et al. 1985; Ohman et al. 1987; Schaller et al. 1999). Since altitude hypoxia increases the sympathoadrenal response to any physical effort (Roberts et al. 1996), it is likely that small differences in physical activity performed at HA would affect the results of the adrenoreceptor studies. Also physical fitness can modulate the exercise-induced regulation of lymphocyte β_2-adrenergic density (Ohman et al. 1987; Schaller et al. 1999).

β_2-Adrenoceptor polymorphism is another factor that could alter the behaviour of the receptor following agonist exposure since it has been demonstrated that the capacity of the β_2-adrenoceptor to down-regulate is enhanced in the polymorphism "gly 16" (Reishaus et al. 1993).

In the study of Antezana et al. (1994) the blood sample for β_2-receptor assay in lymphomonocytes was drawn at altitude whereas our measurements were performed 1 week after returning to sea level. The use in our study of a cell population composed of 85–90% lymphocytes, whose life span is known to be very long, reduces the risk of analysing a cell population different from that exposed to the HA hypoxia. Although a rapid turnover of β_2-adrenoceptors might have masked the modification of receptor density occurring at HA, the recovery of receptor density after exposure to noradrenaline may take several days (Eandi et al. 1984; Skrabal et al. 1986). Consequently, differences in timing of the two β_2-adrenoceptor studies does not wholly explain the discrepancies between them. The regulation of β-adrenoceptor density and function during altitude hypoxia is quite complex, and additional aspects should be considered. For instance, both thyroid hormone (Scarpace and Abrass 1981) and cortisol (Cotecchia and De Blasi 1984), which are increased at extreme altitude, have been shown to up-regulate β-adrenoceptors. In our study, pituitary-thyroid and pituitary-adrenal function were not evaluated; however, the up-regulation induced by thyroid hormones and cortisol could counteract the down-regulation induced by the increase in sympathetic activity, thus resulting in a preserved lymphomonocyte β-adrenoceptor density.

In conclusion, the results from the present investigation, in agreement with most of the relevant literature, suggest that several of the cardiovascular adaptations associated with HA exposure may be mediated or modulated by sympathoadrenal activation. In addition, the adrenoceptor findings indicate that platelet α_2- and lymphomonocyte β_2-adrenergic receptors are regulated differently during HA exposure. In particular, the reduction of the number of α_2-adrenoceptors on circulating platelets may lead to important insights into the mechanisms of the acclimatization process. Whether these modifications are related to the haemodynamic changes observed during prolonged exposure to hypoxia remains to be determined, and further experiments are required to correlate this alteration with post-receptor events. If similar changes are present in other tissues, such as the central nervous system, they may be of importance in the pathogenesis of circulatory adjustments to hypoxia.

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