Carotid baroreflex regulation of vascular resistance in high-altitude Andean natives with and without chronic mountain sickness

Jonathan P. Moore¹, Victoria E. Claydon¹, Lucy J. Norcliffe¹, Maria C. Rivera-Ch², Fabiola Léon-Velarde², Otto Appenzeller³ and Roger Hainsworth¹

¹Institute for Cardiovascular Research, University of Leeds, Leeds LS2 9JT, UK
²Department of Physiological Sciences, Universidad Peruana Cayetano Heredia, Lima 100, Peru
³New Mexico Health Enhancement and Marathon Clinics Research Foundation, Alburquerque, NM87122, USA

We investigated carotid baroreflex control of vascular resistance in two groups of high-altitude natives: healthy subjects (HA) and a group with chronic mountain sickness (CMS), a maladaptation condition characterized by high haematocrit values and symptoms attributable to chronic hypoxia. Eleven HA controls and 11 CMS patients underwent baroreflex testing, using the neck collar method in which the pressure distending the carotid baroreceptors was changed by applying pressures of −40 to +60 mmHg to the chamber. Responses of forearm vascular resistance were assessed from changes in the quotient of blood pressure divided by brachial artery blood velocity. Stimulus–response curves were defined at high altitude (4338 m) and within 1 day of descent to sea level. We applied a sigmoid function or third-order polynomial to the curves and determined the maximal slope (equivalent to peak gain) and the corresponding carotid pressure (equivalent to ‘set point’). The results showed that the peak gains of the reflex were similar in both groups and at both locations. The ‘set point’ of the reflex, however, was significantly higher in the CMS patients compared to HA controls, indicating that the reflex operates over higher pressures in the patients (94.4 ± 3.0 versus 79.6 ± 4.1 mmHg; P < 0.01). This, however, was seen only when subjects were studied at altitude; after descent to sea level the curve reset to a lower pressure with no significant difference between HA and CMS subjects. These results indicate that carotid baroreceptor control of vascular resistance may be abnormal in CMS patients but that descent to sea level rapidly normalizes it. We speculate that this may be explained by CMS patients having greater vasoconstrictor activity at altitude owing to greater hypoxic stimulation of chemoreceptors.

(Received 14 December 2005; accepted after revision 7 June 2006; first published online 8 June 2006)

Corresponding author J. P. Moore; Department of Chemical and Biological Sciences, School of Applied Sciences, University of Huddersfield, Huddersfield HD1 3DH, UK. Email:j.p.moore@hud.ac.uk

Prolonged exposure to high-altitude hypoxia is characterized by a variety of adaptive changes, including erythrocytosis, increased haematocrit and increased haemoglobin concentration (Ward et al. 2000). Chronic mountain sickness (CMS, Monge’s disease) is a condition occurring in some long-term high-altitude dwellers, particularly Andeans, and is characterized by the combination of excessive polycythaemia and a variety of clinical symptoms, including fatigue, pulmonary hypertension and cognitive impairment. This represents a major public health risk for Andean high-altitude natives living at altitudes greater than 3000 m (Léon-Velarde et al. 1993).

Recently we published data indicating that Andean high-altitude natives have an exceptional tolerance to orthostatic stress both at their native altitude of 4338 m and at sea level (Claydon et al. 2005). Tolerance was similar in both healthy and CMS subjects and at both locations despite CMS subjects showing smaller vasoconstrictor responses to orthostatic stress, at least when determined at sea level. We felt that the possible disadvantage of a smaller degree of vasoconstriction might be offset by larger packed...
cell and blood volumes in the CMS subjects (Claydon et al. 2004). However, it is also possible that CMS subjects might have differences in function of the baroreceptor reflexes.

We now report the findings of an additional series of experiments, using the same subjects as in our previous studies, undertaken to investigate the responses of forearm vascular resistance to carotid baroreceptor stimulation. The first aim of these experiments was to determine carotid baroreceptor function in healthy chronically hypoxic high-altitude natives and to determine whether relief of the hypobaric hypoxia by descent to sea level affected the baroreceptor responses. The second objective was to determine whether baroreceptor function in subjects with clinical manifestations of CMS was different from that in the healthy subjects living in the same location. Carotid baroreflex function was assessed from the stimulus–response relationships between carotid pressure and forearm vascular resistance, by determining the maximal slope (equivalent to peak gain) and the corresponding mean carotid pressure (equivalent to ‘set point’).

Methods

Subjects

We recruited 22 male high-altitude natives from Cerro de Pasco, Peru (altitude 4338 m; barometric pressure, 450 mmHg); 11 were known to be suffering from CMS and 11 were apparently normal. None of the subjects had been to low altitude within the 6 months prior to the study and none had worked as a miner. The volunteers were from the same cohorts that took part in our recently reported studies on orthostatic stress (Claydon et al. 2004, 2005) and cerebrovascular responses (Norcliffe et al. 2005). On arrival at the laboratory in Cerro de Pasco, subjects gave detailed clinical histories and provided informed written consent; none of the volunteers was taking prescribed medication for a cardiovascular or respiratory disorder. Peripheral haematocrit was measured, and chronic mountain sickness scores were calculated using the scoring system based on the presence and severity of the 10 most common clinical symptoms and signs of CMS (L`eon-Velarde et al. 2003). The 11 presumed normal subjects had haematocrits < 60% and were categorized as high-altitude normal subjects (HA controls; mean age, 39.3 ± 2 years; haematocrit, 53.6 ± 1.2%). Eleven subjects with haematocrits > 65% were categorized as CMS patients (age, 43.1 ± 2 years; haematocrit, 67.8 ± 2.0%). High CMS scores were associated with subjects who had high haematocrits: 19 ± 5 for CMS patients versus 7 ± 4 for HA controls. All subjects gave their informed written consent, and the study was approved by the Research Ethics Committees of the Universidad Peruana Cayetano Heredia and the Leeds Teaching Hospitals NHS Trust. All procedures conformed to the Declaration of Helsinki (2004).

Measurements

Subjects sat comfortably, and the monitoring devices were fitted. The right hand was supported at heart level, and a photoplethysmographic device (Portapres model 2, TNO-TPD Biomedical Instrumentation, Amsterdam, Netherlands) was positioned on the middle finger. This enabled beat-to-beat estimation of arterial blood pressure, which was checked at regular intervals against brachial arterial pressure, measured using an automated sphygmomanometer (Hewlett Packard 78352C, Boeblingen, Germany) on the opposite arm. Forearm blood flow velocity was determined using a pulsed-wave Doppler system (T2-Dop, DWL Elektronische System GmbH, Sipplingen, Germany) with a 4 MHz probe positioned over the brachial artery at or near the antecubital fossa. The probe was positioned to give the strongest signal with the smallest angle of insolation, and it was then firmly clamped in position, with particular care being taken to ensure that the angle with the brachial artery remained constant during each experiment. In addition, we continuously monitored heart rate and peripheral oxygen saturation using bipolar leads and finger pulse oximetry, respectively (Hewlett Packard 78352C). All variables were recorded on a personal computer via a data acquisition program (Windaq, Dataq Instruments, Akron, OH, USA).

Carotid baroreceptor tests

With the subjects in the sitting position and after a 15 min rest period, changes in the stimulus to carotid sinus baroreceptors were effected by changing the extramural carotid pressures by applying suction or pressure to the skin overlying the sinuses. Negative pressures were applied using a lead chamber similar to that described by Eckberg et al. (1975). The chamber was moulded to fit the subject from the lower border of the mandible to the upper border of the chest and to the posterior neck muscles. To apply positive pressures we used paired chambers as described by Kelly et al. (1993, 1996). These are small chambers made from a thermoplastic material and were of a range of sizes to fit various shapes of neck. Pressure in the chamber(s) was recorded using an automated sphygmomanometer (Hewlett NV300, Numatic, Beaminster, UK). The chambers were connected via a valve to a negative/positive pressure source (Henry NV300, Numatic, Beaminster, UK). With subjects seated upright, tests of baroreceptor responses were performed by setting the pressure to the required level, by use of a variable voltage control, then opening the valve while the neck chamber(s) were held open.
in place. Baroreceptor stimulus–response relationships were determined by changing the pressure in the neck chamber(s) in 20 mmHg steps from −40 to +60 mmHg, then in reverse order. Each pressure was maintained for 20 s and was restored to atmospheric between each step.

Experimental procedure

Subjects were studied first at high altitude in Cerro de Pasco (CP), Peru. They were instructed to abstain from caffeine-containing beverages from the evening before the tests. Tests of carotid baroreceptor function were performed as described above. One week later, the subjects descended to sea level in Lima, Peru, where they were reassessed within 24 h of arrival under similar experimental conditions. Tests of baroreceptor function were undertaken at the same time of day at both locations in around two-thirds of the volunteers.

Data analysis

Forearm vascular resistance was calculated as mean arterial pressure divided by mean blood flow velocity. We analysed the average values of vascular resistance for consecutive periods of five cardiac cycles for the duration of the stimulation period. Each value of vascular resistance was calculated as a percentage of the average value for the 10 cardiac cycles preceding the onset of stimulation. The maximal percentage change was used for subsequent analyses. Transmission of pressure from the chamber(s) to the carotid sinus was assumed to be 100%, and the carotid sinus pressure was estimated as mean arterial pressure (estimated at the level of the sinus) minus chamber pressure.

Values of vascular resistance were plotted against carotid sinus pressure, and the points were fitted with either a sigmoid function or third-order polynomial, depending upon which curve best fitted the data (GraphPad version 4.0, GraphPad Software Inc., San Diego, CA, USA). If a response could not be fitted to either function, the test was considered unsuccessful and not submitted to further analysis. Differentiating the stimulus–response curves determined the maximum slopes, and these were taken as an indicator of maximum baroreflex gain. The carotid sinus pressures corresponding to the maximal slopes were termed ‘set points’, and these values were used to determine possible resetting of the baroreflex. We do not report the absolute values of vascular resistance because, although conditions such as probe angle and positioning were kept constant during each test, they may not have been the same for tests on different days. All of the data were normally distributed, and averaged values are presented at all times as means ± s.e.m. Each volunteer acted as his own control for tests, and within-group comparisons (CMS patient or HA control) were performed using Student’s paired t tests. Between-group comparisons were performed using Student’s unpaired t tests. Statistical significance was accepted at P < 0.05.

Results

Resting arterial oxygen saturation, blood pressure and heart rate

Values of arterial oxygen saturation (S_{aO2}), mean systolic and diastolic arterial pressures and heart rate in 11 HA controls and 11 CMS patients at both locations (CP and Lima) are given in Table 1. Oxygen saturations in CMS patients were significantly lower at both locations compared to HA controls. As expected, the values for both groups in Lima were significantly higher than those in CP. Resting values of systolic blood pressure were not significantly different between the two groups or at the different locations. Diastolic pressures tended to be lower at sea level, significantly so for CMS patients (P < 0.05). Resting heart rate was significantly higher in CMS patients than in HA subjects at altitude (P < 0.05). Heart rate in both groups was significantly less at sea level compared to altitude, but was not significantly different between groups.

Carotid baroreflex function

Satisfactory stimulus–response curves were obtained from 10 HA control and 10 CMS patients for tests in Cerro de Pasco (CP), and from 10 HA control and 11 CMS patients for tests in Lima. Consequently, baroreflex
function was successfully studied in 9 HA and 10 CMS volunteers at both locations. Examples of data showing the relationships between forearm vascular resistance and carotid pressure, in a CMS patient and in a healthy HA control, are summarized in Fig. 1. The values of the maximal gains and ‘set points’ calculated from the stimulus–response relationships of both groups at both locations are listed in Table 2.

Baroreflex control of forearm vascular resistance at high altitude (CP). The maximal gains were not significantly different between the two groups. In contrast, the ‘set points’ were significantly higher in CMS patients (94.4 ± 3.0 versus 79.6 ± 4.1 mmHg; \(P < 0.01\), Student’s unpaired \(t\) test).

The effects of descent to low altitude (Lima) on carotid baroreflex function. For tests of carotid baroreflex function in Lima, there were no significant differences between groups in the values of the maximal gain or ‘set point’. Descent to sea level had no consistent effect on the maximal gain for 10 CMS patients and 9 HA controls tested at both locations. However, in CMS patients tested at both locations, the ‘set point’ was significantly lower in Lima than in CP (e.g. Fig. 1). The mean values were 82.8 ± 4.1 and 94.4 ± 3.0 mmHg, respectively (\(P < 0.01\)). In contrast, in HA subjects tested at both locations descent to sea level had no significant effect on the ‘set point’.

Discussion

The aim of the present investigation was to provide insight into baroreflex control of vascular tone in healthy high-altitude dwellers and to determine any possible differences in subjects with the common maladaptation condition, chronic mountain sickness. The main novel findings of our research are: (1) when tested in their high-altitude hypoxic environment, the ‘set point’ of the relationship between carotid baroreceptor pressure and peripheral vascular resistance was significantly higher in the natives with CMS compared to healthy high-altitude controls; and (2) rapid descent to low altitude significantly lowered the ‘set point’ of the relationship in CMS patients, but not in healthy high-altitude natives. Thus, when tested at low altitude, the ‘set point’ of the relationship in CMS patients became not significantly different from that in HA controls. Maximal baroreflex gains were similar in both groups and at both altitudes.

The variable neck pressure technique is widely acknowledged as an invaluable research tool for the investigation of carotid baroreflex function in human subjects (Fadel et al. 2003). The technique, however, does have its limitations. One of these is that the carotid and other baroreceptors are not isolated from the responses. Thus, any responses resulting from stimulation of the carotid baroreceptors would be buffered and may be smaller than expected. The effect of buffering can be minimized by assessing responses soon after application of the stimulus. However, it is important that the duration of the stimulation period is sufficiently long to allow for the latency of the reflex. Recent observations by our group indicate that the latency of vascular resistance responses to a change in carotid pressure is a little longer than the time...
completed in 10 CMS patients and 9 HA subjects. Tests at both locations were successfully repeated within 24 h of descent to Lima: CMS patients, 84.2 ± 3.9 mmHg (n = 10); HA subjects, 77.4 ± 5.3 mmHg (n = 10). Furthermore, there was no significant difference in the slope in CP or in Lima. Within-group comparisons indicate that descent to Lima significantly reduced the set point in 10 CMS patients (82.8 ± 4.1 mmHg) but had no effect in 9 HA subjects (79.2 ± 5.6 mmHg). Note that 22 volunteers were recruited for the study. Satisfactory results were obtained in 10 CMS patients and 10 HA subjects in CP, and in 11 CMS patients and 10 HA subjects in Lima. Tests at both locations were successfully completed in 10 CMS patients and 9 HA subjects.

Our results showed that under conditions of high-altitude hypobaric hypoxia there was no consistent difference in the ‘sensitivity’ (maximal slope) of the carotid baroreceptor–vascular resistance reflex between the two groups of high-altitude natives. In addition, the sensitivities were not significantly affected in either group by relief of the chronic hypoxia following descent to sea level. These results differ from a previous report (Keyl et al. 2003) in which CMS patients were reported to have a lower spontaneous baroreflex sensitivity than healthy controls, suggesting an impairment of cardiovascular regulation. In that study the authors also showed that this impairment was alleviated by temporary administration of supplemental oxygen. However, it should be noted that Keyl et al. (2003) examined only the cardiac responses and not the vascular responses which we were examining. We did not attempt to study the cardiac component of the baroreceptor reflex because it necessitates making measurements during held expiration (Eckberg, 1976), and this would have required an uncomfortably long protocol for the experiments described here. Furthermore, we believe that it is the control of vascular resistance that is of major importance in blood pressure control, with cardiac responses being relatively unimportant (Cooper & Hainsworth, 2002; Ogoh et al. 2002, 2003).

Our results, however, are compatible with those of a study by Halliwill & Minson (2002) in which they tested for the maximal blood pressure change, i.e. 10 s (Gulli et al. 2005). Hence, a neck-collar stimulation period lasting for 15 s seems appropriate, and this is what we used for the present study. We then determined the maximal responses of forearm vascular resistance during this period and used these in subsequent analyses.

Responses of vascular resistance in the forearm were determined from changes in the ratio of brachial artery velocity to mean arterial pressure. This has been used in previous studies (Brown & Hainsworth, 2000; Cooper & Hainsworth, 2001, 2002) and it provides a continuous and reliable indicator of changes in vascular resistance, provided that the angle of the Doppler probe remains constant in relation to the artery. The maximal responses of forearm vascular resistance were plotted against calculated carotid sinus pressure on the assumption that all the applied positive or negative pressures are transmitted to the sinus. This is unlikely, however, and previous studies have indicated that 89% of the neck pressure and around 83% of neck suction is actually transmitted to the area of the carotid sinus (Eckberg 1976; Querry et al. 2001). Our assumption of 100% transmission is therefore likely to result in a small underestimate of the maximal gain, but it would have very little effect on the value of ‘set point’. Furthermore, there is no reason to believe that the pressure transmission would be different in the two groups or at the two locations, and so the effect should not invalidate any of our comparisons.

A further methodological limitation that should be mentioned relates to the order of the studies. Ideally we should have randomized the order of the high- and low-altitude tests. However, one of the criteria we adopted was that none of the subjects had been to low altitudes for at least 6 months before the study, so randomization was not practicable.
the effect of breathing a hypoxic gas mixture in healthy sea-level natives and found that this had no significant effect on the sensitivities of baroreflex regulation of either muscle sympathetic nerve activity or heart rate.

In contrast to our findings for baroreflex sensitivity, we did find that the ‘set point’ was significantly higher in CMS patients compared to healthy HA control subjects, but only when studied at altitude. It is interesting to note that when the CMS patients were tested at sea level, the ‘set point’ was significantly lower compared to that in the same group studied at their resident altitude. In contrast, whilst the ‘set point’ of the relationship in the HA controls tended to be lower at sea level, it was not significantly changed between the locations. Furthermore, the values for ‘set points’ in CMS patients and HA control subjects in Lima were not significantly different.

There are several possible explanations for the higher ‘set point’ in CMS patients at altitude compared to their values at sea level and to those of the healthy subjects at both locations. First, the haematocrit values (by selection) were significantly higher in the CMS subjects. Thus, the effect of the greater packed cell volume on blood viscosity and forearm vascular resistance may have caused the ‘set point’ to be different between the two groups when studied at high altitude. In unacclimatized newcomers to altitude, there is haemoconcentration through loss of plasma volume (Jain et al. 1980). It is not known whether there is a similar haemodilution from plasma volume expansion in high-altitude natives who descend to sea level. However, if this were to occur, haemodilution might be expected to have a greater effect on the blood viscosity, and thus vascular resistance, of the CMS subjects, and this could partly explain the ‘normalization’ of the ‘set point’ in these subjects at sea level. It should be pointed out, however, that although haematocrit was lower following descent, it decreased by approximately 4%, possibly due to previous blood sampling. There were no significant changes in plasma volume in either group upon descent to sea level (Claydon et al. 2005). Furthermore, descent to sea level was characterized by a decrease in resting blood flow velocity and increased vascular resistance. We feel, therefore, that the small changes in haematocrit could not be the main reason for the changes in ‘set point’ in the CMS group.

A second, perhaps more likely, explanation for the observed difference in ‘set point’ at high altitude, and its normalization at sea level, may be related to the effects of arterial hypoxaemia on sympathetic vasomotor activity. Both groups had low arterial saturations at high altitude and, as previously reported (León-Velarde et al. 1993; Sun et al. 1996; Keyl et al. 2003), arterial oxygen saturation was even lower in the CMS group. It is well known that sympathetic discharge to muscle vascular beds is increased by hypoxia (Saito et al. 1988; Somers et al. 1989; Duplain et al. 1999; Halliwill & Minson, 2002). It is likely that there would have been a greater sympathetic activation in the more hypoxaemic CMS patients, and this may have contributed to the altered carotid baroreflex function. This hypothesis is supported by the observation that resting noradrenaline and adrenaline levels are greater in CMS patients than in HA normal subjects (Antezena et al. 1995), suggesting greater levels of sympathetic activity.

Descent to sea level relieved the hypoxaemia in both groups, and coincided with normalization of baroreflex function in CMS patients, i.e. baroreflex control of vascular resistance is reset to lower pressure. It is likely that the level of sympathetic activity in both groups would have been less at sea level; this is reflected in the lower heart rates and diastolic blood pressures. However, to test whether or not a greater reduction in sympathetic vasomotor activity could explain the normalization of the ‘set point’ in CMS subjects, it would have been necessary to investigate sympathetic efferent activity directly, and this was beyond the scope of this study. However, a recent study by Gamboa et al. (2006) found that plasma catecholamines in high-altitude natives from Cerro de Pasco were significantly reduced within 4 h of descent to Lima. The effect of acute inhalation of oxygen-rich air on baroreflex function was not tested at high altitude, but in the only previous study of baroreflex function in high-altitude natives, Keyl et al. (2003) observed that temporary administration of supplemental oxygen, as well as slow-frequency breathing, both of which elevated arterial oxygen saturation, normalized cardiac baroreflex function in CMS patients.

To our knowledge this is the first study which has examined vascular responses to carotid baroreceptor stimulation in these two groups of subjects and the first study to have undertaken baroreflex function tests following rapid descent to sea level. We have, however, recently reported, using similar techniques, that the carotid baroreceptor–vascular resistance relationship of healthy lowland dwellers breathing a hypoxic, hypercapnic gas is also displaced to higher pressures (Cooper et al. 2004). Thus CMS highlanders appear to be maladapted to hypoxia in a similar manner to healthy lowlanders, whereas baroreflex function in healthy highlanders is unaffected by hypoxia. However, there are several differences in these two studies. In the healthy lowland dwellers, the hypoxia was normobaric and only acutely applied. Genetic factors are also likely to contribute, and it would be of interest to study some Andean men who had been living at low altitude since birth, but were genetically similar to the volunteers in the present study.

In summary, this study has shown that baroreflex function in healthy Andean high-altitude natives is not altered following descent to sea level. In CMS patients, with high haematocrit, low S\text{\textsubscript{aO}_2\text{}} and significant signs and symptoms of CMS, however, the ‘set point’ when measured at altitude was significantly higher than in normal subjects.
In these patients within 24 h of a rapid descent to sea level, baroreflex function changed, as indicated by baroreceptor resetting to lower pressures. This change may have resulted partly from reduced central sympathetic drive following the increase in $S_{\mathrm{O}_2}$; however, other effects, for example haemodilution, may also have contributed.

References


Author’s present address

J. P. Moore: Department of Chemical and Biological Sciences, School of Applied Sciences, University of Huddersfield, Huddersfield HD1 3DH, UK.