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Cardiovascular effects of 8 h of isocapnic hypoxia with and without β-blockade in humans

Christine Clar, Keith L. Dorrington, Marzieh Fatemian and Peter A. Robbins*

University Laboratory of Physiology, University of Oxford, Parks Road, Oxford OX1 3PT, UK

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This study seeks to confirm the progressive changes in cardiac output and heart rate previously reported with 8 h exposures to constant hypoxia, and to examine the role of sympathetic mechanisms in generating these changes. Responses of ten subjects to four 8 h protocols were compared: (1) air breathing with placebo; (2) isocapnic hypoxia (end-tidal $P_{O_2} = 50$ mmHg) with placebo; (3) isocapnic hypoxia with β-blockade; and (4) air breathing with β-blockade. Regular measurements of heart rate and cardiac output (using ultrasonography and N_2O rebreathing techniques) were made with subjects seated in the upright position. The sensitivity of heart rate to rapid variations in hypoxia (G_{HR}) and heart rate in the absence of hypoxia were measured at times 0, 4 and 8 h. No significant progressive effect of hypoxia on cardiac output was detected. There was a gradual rise in heart rate with hypoxia of 11 ± 2 beats min^{-1} in the placebo protocol and of 10 ± 2 beats min^{-1} in the β-blockade protocol over 8 h, compared to the air breathing protocols. The rise in heart rate was progressive ($P < 0.001$) and accompanied by progressive increases in both G_{HR} ($P < 0.001$) and heart rate measured in the absence of hypoxia ($P < 0.05$). No significant effect of β-blockade was detected on any of these progressive changes. We conclude that sympathetic mechanisms that act via β-receptors play little role in the progressive changes in heart rate observed over 8 h of moderate hypoxia. *Experimental Physiology* (2000) **85.5**, 557–565.

The cardiovascular system exhibits responses to hypoxia that occur over a wide range of different time scales. The present study focuses mainly on those responses that occur over a period of hours, after the initial acute (seconds to minutes) responses are complete. In relation to this time scale, Dorrington *et al*. (1997) recently demonstrated that there was a progressive rise in cardiac output over an 8 h period of steady hypoxia. This increase in cardiac output was associated with an increase in heart rate, with stroke volume remaining relatively constant. The progressive increase in heart rate over 8 h of hypoxia has also been observed in a separate study from our laboratory (Clar *et al*. 1997), and has been shown to consist of two components. The first of these components is an increase in the sensitivity of heart rate to acute variations in the level of hypoxia. The second component is an increase in 'basal' heart rate under hyperoxic conditions – i.e. there is a component of the increase in heart rate that is not rapidly reversed on exposure to acute hyperoxia.

The progressive increase in heart rate could arise through an increase in sympathetic activity, a decrease in parasympathetic activity, or through a direct effect of hypoxia on the heart. In relation to an increase in sympathetic activity, a number of researchers have shown progressive increases in plasma or urinary catecholamine levels in humans during prolonged exposures to high altitude (Cunningham *et al*. 1965; Mazzeo *et al*. 1994, 1998). In this study, we examine the hypothesis that sympathetic mechanisms contribute to the heart rate response to 8 h of isocapnic hypoxia. In particular, we describe the influence of β-adrenergic blockade on cardiovascular changes observed during 8 h of isocapnic hypoxia. Heart rate, cardiac output and systemic arterial blood pressures were measured at regular intervals during the hypoxic exposure in the upright seated position. In addition to these measurements, the heart rate response was examined in more detail to distinguish between increases in the sensitivity of heart rate to acute variations in hypoxia, and increases in heart rate that persist under conditions of acute hyperoxia. The data for this study were gathered contemporaneously with a study of the effects of β-blockade on changes in respiratory function over 8 h of isocapnic hypoxia (Clar *et al*. 1999).

METHODS

Subject

Ten healthy subjects (6 male, 4 female) aged between 20 and 28 years participated in this study. None of them had a history of respiratory or cardiovascular disease. All subjects gave informed consent to the study. The study had been approved by the Central Oxford Research Ethics Committee and was performed according to the Declaration of Helsinki. Subjects attended in the first half of the morning, following their usual breakfast and a short journey to the laboratory.

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* Corresponding author: peter.robbins@physiol.ox.ac.uk

Protocols

The protocols were designed so as to allow us to compare the effects of hypoxia with and without β-blockade. The volunteers were subjected to four protocols on 4 different days (in varied order, with protocols separated by at least a week). Female subjects were only studied during the first 2 weeks of their menstrual cycle unless they were taking oral contraceptive tablets, so as to avoid variations between protocols that might be due to variations in the levels of circulating progesterone (Dempsey *et al*. 1986; Dutton *et al*. 1989).

Each protocol lasted 8 h. The four protocols used were as follows. (1) **Protocol C-C**. Air breathing control, where a placebo tablet replaced the propranolol tablet given in protocols IH-P and C-P. (2) **Protocol IH-C**. Isocapnic hypoxia, where end-tidal P_{O_2} (P_{ET,O_2}) was held at 50 mmHg, and end-tidal P_{CO_2} (P_{ET,CO_2}) was maintained at the subject's normal pre-hypoxic value, and where a placebo was given as indicated above. (3) **Protocol IH-P**. Isocapnic hypoxia as in protocol IH-C, with β-blockade. Four 80 mg doses of oral β-blocker (propranolol) were given 8-hourly, starting about 18 h before the beginning of the hypoxic exposure. (4) **Protocol C-P**. Air breathing control, where propranolol was given as in protocol IH-P.

During these protocols, heart rate, systemic arterial blood pressure and cardiac output were measured at times 0, 1, 2, 4, 6 and 8 h. At times 0, 4 and 8 h the sensitivity of heart rate to acute variations in the level of hypoxia was determined along with heart rate under conditions of acute hyperoxia. The P_{ET,O_2} profile used for these measurements is depicted in Fig. 1. A 5 min lead-in period at a steady P_{ET,O_2} of 100 mmHg was followed by six square waves with P_{ET,O_2} alternating between 50 and 100 mmHg, with each gas level being maintained for 1 min. After the last step, the P_{ET,O_2} was increased to 300 mmHg and maintained at that level for 5 min. P_{ET,CO_2} was kept at 1–2 mmHg above the subject's normal air breathing value for the duration of these measurements.

Gas control

Two techniques were used for controlling the subjects' end-tidal gas compositions. For the 8 h exposures, the subjects were inside a specially designed experimental chamber in which the atmosphere could be regulated to achieve the desired end-tidal gas composition (Howard *et al*. 1995). For the measurements at 0, 4 and 8 h that required the rapid variation in P_{ET,O_2} of Fig. 1, a dynamic end-tidal forcing system (Robbins *et al*. 1982; Howson *et al*. 1987) was used that required the subjects to breathe through a mouthpiece while wearing a noseclip. Further details of the gas regulation techniques for the present study are given in Clar *et al*. (1999).

Cardiovascular measurements

The measurements at 0, 1, 2, 4, 6 and 8 h of systemic arterial blood pressure, cardiac output and heart rate were carried out in the chamber with subjects seated in the upright position. Systemic blood pressure was measured using standard manual sphygmomanometry. Cardiac output was estimated non-invasively using both pulsed Doppler ultrasonography and nitrous oxide (N_2O) rebreathing. Heart rate was obtained from the velocity profiles associated with the Doppler ultrasonography.

For the ultrasonic assessment of cardiac output, a 2 MHz Pedof (Vingmed) pulsed Doppler system combined with a Doptek spectrum analyser (Model 9000, Chichester, UK) was used. The velocity of blood in the proximal ascending aorta was measured with the ultrasound probe positioned in the suprasternal notch so that the angle of insonation was close to 0 deg. To obtain an optimal signal, the sample volume (border nearest the probe) was typically positioned about 1 cm above the aortic valve. The technique has been described in detail by Innes (1987). The spectrum analyser incorporated functions to calculate stroke distance and heart rate from the intensity-weighted mean velocity profile. Data collected over 5 s were used to derive a value for cardiac output. As we were interested in relative changes in cardiac output over time and not in absolute changes, an exact measurement of the aortic diameter was not deemed necessary, and a constant diameter of 2.5 cm was assumed for the purpose of converting stroke distance to stroke volume. At each time point, two measurements of cardiac output by Doppler ultrasound were averaged to obtain the reported result.

For the N_2O rebreathing assessment of cardiac output, the subjects rebreathed for 30 s from a 4 l anaesthetic bag which was filled with a gas mixture of approximately 20% N_2O and 5% CO_2 in O_2. The volume of gas in the bag was equivalent to 60% of the subject's predicted functional residual capacity, which was obtained from their height and age according to a standard formula (Qanjer, 1983). The subjects were instructed to inspire the whole contents of the bag with every breath, and to breathe regularly at a rate of about 22 breaths per minute as indicated by an electronic bleep. During this period, gas was sampled continuously from a catheter placed inside the dead space of the mouthpiece, and analysed for N_2O using a Datex gas analyser (Normocap 200 OXY, Instrumentation Corp., Helsinki, Finland). The data for N_2O were collected on a PC running a data acquisition programme (Picolog, Pico Technology Ltd, Hardwick, UK). For analysis, the data collected were plotted, end-tidal values of N_2O were identified manually, and a bi-phasic exponential curve was fitted to the end-tidal values using non-linear regression. Cardiac output was calculated according to the equation derived by Hook & Meyer (1982). At each time point, two measurements of cardiac output by N_2O rebreathing were averaged to obtain the reported result.

For the measurements made at 0, 4 and 8 h of the sensitivity of heart rate to acute exposures to hypoxia and of heart rate in acute hyperoxia, heart rate was derived from an ECG (Rigel Cardiac Monitor 302, Morden, UK). The occurrence of each QRS complex was recorded in a data file, and this information was used to derive values for heart rate on a beat-by-beat basis. Data from the last 3 min of the 5 min period of hyperoxia were used to obtain the measured value of heart rate in hyperoxia.

Modelling of the heart rate response to hypoxia

In order to obtain numerical estimates of the sensitivity of heart rate to acute variations in hypoxia, a model of the heart rate response to acute hypoxia was fitted to the data. We assumed that, once the acute response to hypoxia was complete, heart rate (HR) could be represented as the sum of a baseline heart rate to be expected at 100% saturation ($HR_{100\%}$) plus a component attributable to the reduction in saturation (S) below 100%: $HR = HR_{100\%} + G_{HR}(100 - S)$, where G_{HR} is the sensitivity of heart rate to a reduction in saturation.

Under the conditions of dynamic hypoxic stimulation that we have been studying, we also have to take into account the time HR takes to move towards a new steady-state value when the saturation is changed, and the time it takes blood with a given saturation in the lungs (that can be derived from P_{ET,O_2}) to reach the target chemosensitive organ (for example the carotid bodies) where the stimulus acts to produce the physiological response. A simple differential equation to describe this process is:

$$\tau \frac{dHR}{dt} + HR = HR_{100\%} + G_{HR}(100 - S(t - t_d)),$$

where τ is a time constant representing the rate at which the heart rate changes and t_d is the delay time for the blood to travel from the lungs to a chemosensitive organ. By assuming that $S(t - t_d)$ remains constant from the beginning to the end of individual heart beats, the equation may be solved to yield HR for heart beat i, as a function of the input (S), the parameters of the model, and the value of HR for heart beat $i - 1$.

$$HR_i = [HR_{100\%} + G_{HR}(100 - S(t - t_d))] - [HR_{100\%} + G_{HR}(100 - S(t - t_d)) - HR_{i-1}]\exp(-(t_i - t_{i-1})/\tau),$$

where t_i is the time at the ith heart beat. The parameters of this model ($HR_{100\%}$, G_{HR}, τ and t_d) were estimated by non-linear regression using the Numerical Algorithms Group (Oxford, UK) Fortran library routine E04FDF to minimise the sum of squares of the residuals. S was calculated from the measured P_{ET,O_2} values using the haemoglobin dissociation function as described by Severinghaus (1979).

Statistical analysis

Except where otherwise indicated, tests of statistical significance were undertaken using analysis of variance. A correction for repeated measures was made as appropriate, based on the approach of Greenhouse & Geisser (Crowder & Hand, 1990). Fixed factors were time, presence or absence of hypoxia (hypoxia), and presence or absence of drug (drug). Subjects were treated as a random factor. A particular term of interest in the analysis of variance was the interaction between drug, hypoxia and time, to address the question of whether the drug significantly altered the response of the respective variables over time to hypoxia. In the case of the measurements taken in the chamber (heart rate during hypoxia and cardiac output), the pre-hypoxic air breathing values at $t = 0$ were excluded from the analysis, and only values between 1 h and 8 h were compared. This was in order to ensure that any significant changes over time that might be detected would relate to slow changes over time under constant conditions rather than to differences between hypoxic and non-hypoxic measurements.

Results are given in the text as means ± S.E.M., unless otherwise specified.

RESULTS

Subjects

All ten subjects completed this study successfully, although some suffered from headaches during the second half of the hypoxic exposure.

End-tidal values before and during protocols

Values for air breathing P_{ET,CO_2} at the beginning of the experimental day were slightly but significantly lower (1.5 ± 0.4 mmHg, $P < 0.01$) in the presence of propranolol than when placebo had been taken. Figure 2 illustrates the mean values for P_{ET,O_2} and P_{ET,CO_2} obtained in the chamber for the four protocols. This figure suggests that P_{ET,O_2} and P_{ET,CO_2} were very constant throughout the chamber exposure. Mean values of P_{ET,O_2} were 109.1 ± 3.1 mmHg in protocol C-C (means of 5 min averages of breath-by-breath end-tidal values ± S.D.), 52.3 ± 0.6 mmHg in protocol IH-C, 52.5 ± 0.8 mmHg in protocol IH-P, and 108.7 ± 5.9 mmHg in protocol C-P. These measurements corresponded with values of arterial saturation from pulse oximetry of 97.2 ± 0.6% for protocol C-C, 87.0 ± 1.2% for protocol IH-C, 86.7 ± 1.1% for protocol IH-P, and 97.1 ± 0.4% for protocol C-P. Mean values of P_{ET,CO_2} were 38.8 ± 3.3 mmHg in protocol C-C, 38.9 ± 3.7 mmHg (−0.2 ± 0.3 mmHg difference from target value) in protocol IH-C, 37.5 ± 4.0 mmHg (−0.2 ± 0.8 mmHg difference from target value) in protocol IH-P, and 37.8 ± 4.8 mmHg in protocol C-P.

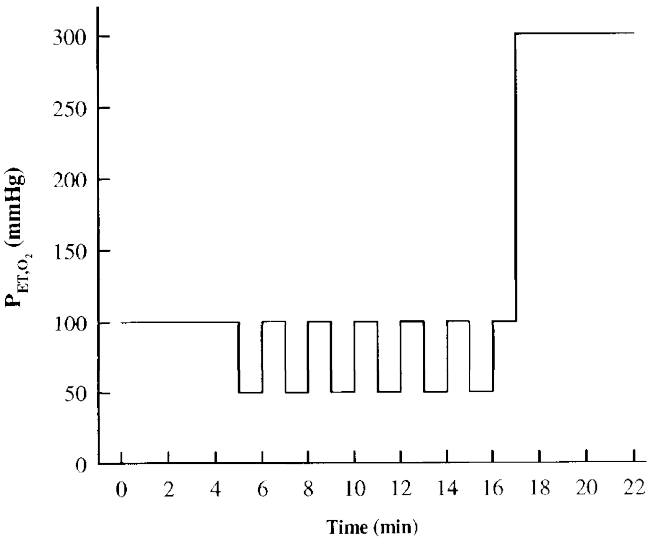

Figure 1

P_{ET,O_2} profile used to assess the sensitivity of heart rate to acute variations in hypoxia and heart rate during hyperoxia. P_{ET,CO_2} was held at 1–2 mmHg above the subject's normal air breathing value for the whole protocol.

Systemic arterial blood pressure

Figure 2 shows the mean values for systolic and diastolic blood pressure for the different protocols. At the beginning of the experimental day, arterial blood pressure was noted to be significantly lower after β-blockade than with placebo (diastolic pressure lower by 9 ± 2 mmHg, systolic pressure by 12 ± 2 mmHg, $P < 0.005$).

Cardiac output

Measurements for cardiac output obtained both by pulsed Doppler ultrasound and N_2O rebreathing are shown in Fig. 2. In most subjects the Doppler cardiac output measurements correlated well with the N_2O rebreathing cardiac output measurements ($P < 0.01$ for 8 out of 10 subjects, Pearson correlation coefficient). However, the Doppler measurements were consistently lower than the N_2O rebreathing measurements ($P < 0.001$, Student's paired t test).

The figure shows that for both types of cardiac output measurement, values obtained during β-blockade were significantly lower than values obtained when placebo was given ($P < 0.01$, 0.5 ± 0.1 l min^{-1} lower for Doppler data, 0.8 ± 0.1 l min^{-1} lower for N_2O rebreathing data). Hypoxia elevated cardiac output by 0.5 ± 0.1 l min^{-1} compared with control for the Doppler data ($P < 0.05$), but this was not evident for the N_2O data (where all measurements were made using N_2O in a hyperoxic gas mixture).

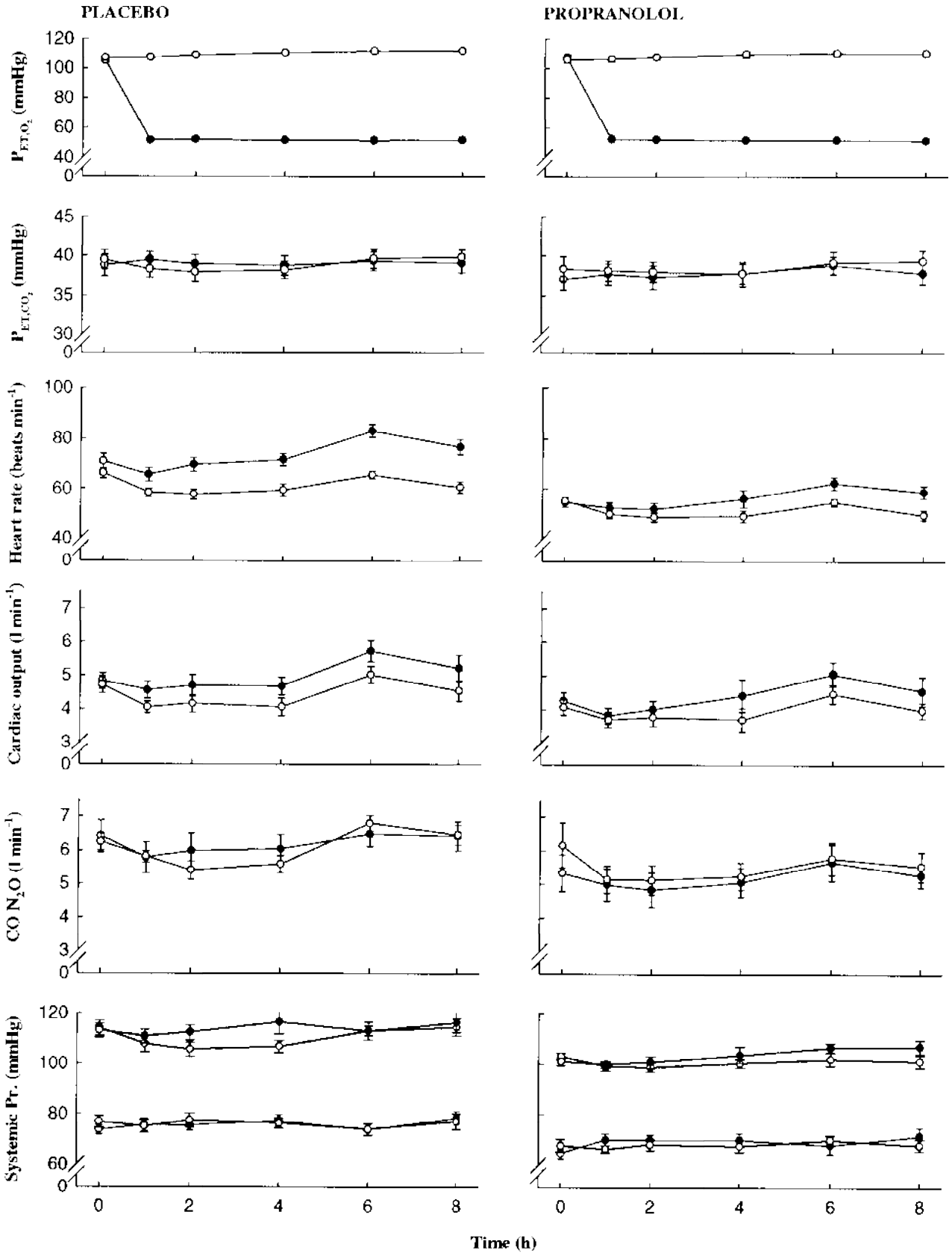

Figure 2. For legend see facing page.

There was a clear variation over time in the values for cardiac output which was similar for all protocols ($P < 0.001$, both Doppler and N_2O data). Particular points to note are, first, the relatively high values at $t = 0$ suggesting that the subjects were not completely settled at the beginning of the protocol (values at $t = 0$ were excluded from the ANOVA for the data obtained within the chamber). Secondly, rather high values were observed at 6 h, which was the first measurement after lunch (served after the 4 h measurement). The plot for the Doppler measurements of cardiac output for propranolol suggests that there was a progressive effect of the hypoxic exposure as evidenced by the progressive widening of the difference between the values recorded during air breathing and the values recorded during hypoxia. However, this was not apparent in the placebo data and overall no significant interaction between hypoxia and time was detected for either the Doppler data or the N_2O rebreathing data.

Heart rate during hypoxia

Figure 2 shows the mean values for the heart rate response measured in the chamber. The plot shows that β-blockade was effective, as propranolol substantially reduced heart rate both in the hypoxic and in the air breathing control protocols (reduction by 13 ± 1 beats min^{-1}, $P < 0.001$). To examine the difference in heart rate (ΔHR) between the protocol with hypoxia and the air breathing control protocol for both pharmacological conditions, normalised ΔHR values are plotted in Fig. 3 as a function of time for the 8 h experiment. The figure shows that hypoxia increased heart rate progressively in both propranolol and placebo protocols ($P < 0.001$, by an average over 8 h of 10 ± 2 ($18.6 \pm 4.3\%$) and 11 ± 2 beats min^{-1} ($17.1 \pm 3.0\%$), respectively). The rate of increase in heart rate over time during hypoxia was not significantly affected by the drug.

Heart rate sensitivity

Figure 4 shows a typical response to the protocol used for the assessment of the sensitivity of heart rate to acute variations in hypoxia and the heart rate response to hyperoxia. This figure shows a good control of P_{ET,O_2} and P_{ET,CO_2}, and a clear variation in heart rate during the hypoxic square waves. The overall time constant τ for the heart rate response to hypoxia was 8.5 ± 0.6 s. Neither hypoxia nor the drug had a consistent effect on this value. In case of the pure delay t_d, propranolol significantly ($P < 0.05$) lengthened this term from 4.1 ± 0.5 to 6.1 ± 0.7 s.

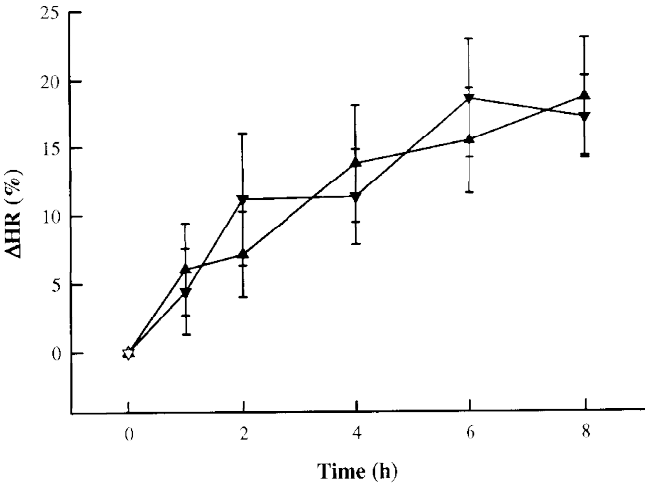

Figure 3

Normalised difference in heart rate (ΔHR) between the hypoxia protocol and the air breathing protocol for each of the pharmacological conditions: placebo (▼) and propranolol (▲). Data were normalised with respect to the $t = 0$ value of each experimental day. Values are means ± S.E.M.

Figure 5 shows the means for three of the variables obtained during these measurements at 0, 4 and 8 h for the four protocols. The top panel shows G_{HR}, the sensitivity of heart rate to acute variations in hypoxia; the middle panel shows $HR_{100\%}$, the calculated value of heart rate at 100% saturation; and the bottom panel shows the measured heart rate during hyperoxia. Considering G_{HR} first, the plots show that G_{HR} increased over time during hypoxia, with and without propranolol, and this effect was significant ($P < 0.005$). Contrary to the impression given by the graph, the drug did not affect the increase in G_{HR} over time significantly. The graph also suggests that propranolol attenuated the control ($t = 0$) values of G_{HR}, and this effect was significant ($P < 0.01$).

Heart rate during hyperoxia

The calculated $HR_{100\%}$ and heart rate measured during hyperoxia essentially represent the same variable, and when comparing values of $HR_{100\%}$ and heart rate measured during hyperoxia, no difference was detected (paired t test). As expected, propranolol significantly decreased both the

Figure 2

End-tidal gas partial pressures and physiological responses recorded in the chamber for the four protocols. Top to bottom: P_{ET,O_2}, P_{ET,CO_2}, heart rate, cardiac output (Doppler technique), cardiac output (N_2O rebreathing), and arterial blood pressure (systolic and diastolic). Left, protocols with placebo; right, protocols with propranolol. ○, values during euoxia; ●, values during hypoxia. Values at $t = 0$ were excluded from the statistical analysis. Values are means ± S.E.M. Significant effects were as follows. Heart rate was reduced by propranolol ($P < 0.001$) and increased progressively over time by hypoxia ($P < 0.001$). This increase was unaffected by propranolol. Cardiac output (Doppler and N_2O measurements) was reduced by propranolol ($P < 0.01$) and varied significantly over time ($P < 0.001$). Blood pressures (systolic and diastolic) were reduced by propranolol ($P < 0.005$).

calculated baseline heart rate at 100% saturation, $HR_{100\%}$ ($P < 0.001$) and the measured heart rate during hyperoxia ($P < 0.001$). The plots show that both $HR_{100\%}$ and heart rate during hyperoxia were higher in the hypoxic protocols than in the air breathing control protocols, and this was significant ($P < 0.05$). The difference between air breathing values and hypoxic values for both variables appeared to increase over time, and this finding was significant for both $HR_{100\%}$ and hyperoxic heart rate ($P < 0.05$). From the plots there was also some suggestion that the drug attenuated the progressive effect of hypoxia, but this was not significant.

Relationship between changes in heart rate and changes in acute ventilatory response to hypoxia

In order to assess whether there was any relationship between the magnitude of G_{HR} and the acute ventilatory response to hypoxia (values from Clar *et al.* 1999), a correlation was performed between the two variables for the $t = 0$ data from protocol C-C. This correlation failed to reach significance ($r = 0.61$, $0.05 < P < 0.10$, Pearson correlation). Similarly, any correlation between the increment in heart rate sensitivity over 8 h of hypoxia (protocol IH-C) and the increment in the acute ventilatory sensitivity to hypoxia over the same period also failed to reach significance ($r = 0.63$, $0.05 < P < 0.10$).

DISCUSSION

In this study, we were unable to show clearly a progressive effect of 8 h of hypoxia on cardiac output. However, there was a progressive increase in heart rate with hypoxia, as has been previously reported (Clar *et al.* 1997; Dorrington *et al.* 1997). The present study found no effect of β-blockade on the progressive increase in heart rate with hypoxia, or on either of its underlying components (G_{HR} and $HR_{100\%}$). These findings suggest that an increase in sympathetic activity does not play a major role in the increase in heart rate that can be observed over 8 h of moderate hypoxia in humans. A secondary finding of the present study was that propranolol does cause a small decrease in G_{HR} under control conditions of no hypoxic exposure.

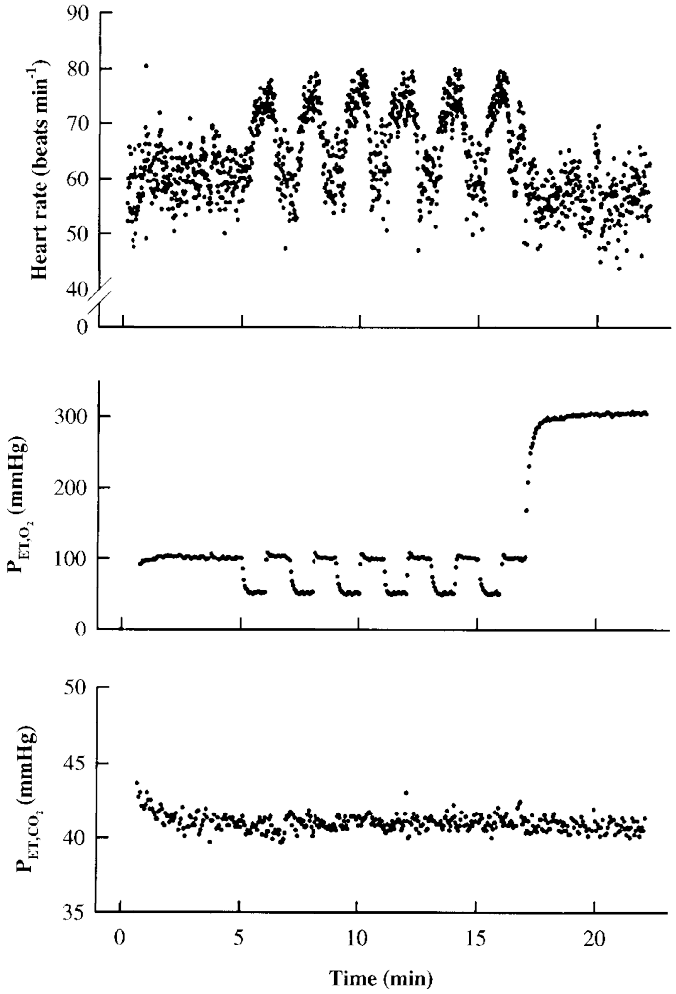

Figure 4

Example assessment of sensitivity of heart rate to acute exposures to hypoxia and of heart rate during hyperoxia. Beat-by-beat data for heart rate (top), breath-by-breath data for P_{ET,O_2} (middle) and P_{ET,CO_2} (bottom).

Methodological considerations

Propranolol is a lipid-soluble non-specific β-adrenergic receptor blocker capable of crossing the blood–brain barrier. Any effects seen could therefore have been brought about through peripheral or central mechanisms, acting at β_1- or β_2-receptors. The dose of propranolol used in this study was the same as that used in various studies of cardiorespiratory responses at altitude (Moore *et al.* 1986, 1987; Hughson *et al.* 1994; Mazzeo *et al.* 1994; Asano *et al.* 1997). Bodem *et al.* (1973) found that propranolol achieved a maximal effect on heart rate in humans at an oral dose of 200 mg per day, subdivided into doses given at 6 h intervals. This observation coupled with the depression of heart rate that was observed in our study (Fig. 2) suggests that the dose of propranolol employed was adequate for effective β-blockade.

Cardiac output

The considerable difference between the absolute values for cardiac output obtained using the N_2O rebreathing technique and Doppler ultrasonography, coupled with the general lack of accuracy associated with indirect techniques, means that the absolute values for cardiac output in this study should be

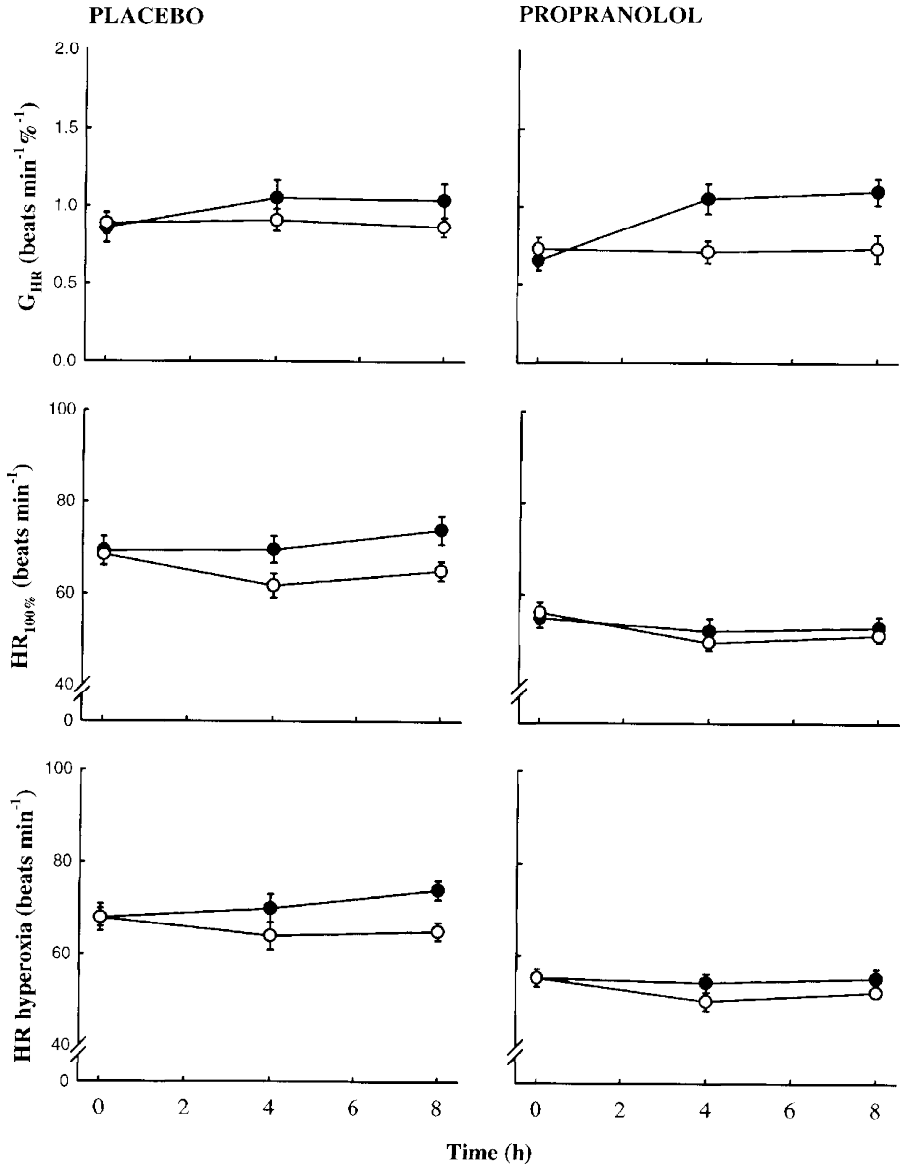

Figure 5

Components of the heart rate response determined using end-tidal forcing for the four protocols. Top, G_{HR}; middle, $HR_{100\%}$; and bottom, measured heart rate during hyperoxia. Left, protocols with placebo; right, protocols with propranolol. ○, protocols involving euoxic exposures; ●, protocols involving hypoxic exposures. Values are means ± S.E.M. Main significant effects: G_{HR} was increased over time by hypoxia ($P < 0.005$) and this increase was unaffected by propranolol. $HR_{100\%}$ and HR during hyperoxia were decreased by propranolol ($P < 0.001$) and increased by hypoxia ($P < 0.05$). This increase was progressive ($P < 0.005$).

treated with considerable caution. However, the good correlation between pulsed Doppler measurements and N_2O rebreathing measurements of cardiac output supports the usefulness of the measurements for detecting changes in cardiac output. A methodological problem associated with the N_2O rebreathing measurements is that they were carried out using a hyperoxic gas mixture, which in itself may have acutely reduced the increase in cardiac output in response to the hypoxic exposure. We will therefore only consider the Doppler measurements of cardiac output further in this section.

In a previous study (Dorrington *et al*. 1997), a gradual increase in cardiac output was observed over an 8 h exposure to the same level of hypoxia as employed in the present study. From the raw data associated with the previous study, the rise in cardiac output between 1 and 8 h can be calculated as 0.98 l min^{-1} (95% confidence interval (CI) 0.36 to 1.34 l min^{-1}, $P < 0.05$, one-sample t test). In the present study, similar significant ($P < 0.05$) rises in cardiac output between 1 and 8 h can be demonstrated for the hypoxic exposures (protocol IH-C, rise of 0.65 l min^{-1}, 95% CI 0.27–1.03 l min^{-1}; protocol IH-P, rise of 0.73 l min^{-1}, 95% CI 0.21–1.25 l min^{-1}). However, in the present study there were also significant rises between 1 and 8 h for the air breathing protocols, and once these changes had been subtracted from the data from the hypoxic protocols, the rises in cardiac output with hypoxia were no longer significant (control protocols, rise of 0.17 l min^{-1}, 95% CI –0.23 to 0.57 l min^{-1}; propranolol protocols, rise of 0.46 l min^{-1}, 95% CI –0.09 to 1.01 l min^{-1}). The previous study lacks these control data with which to correct any apparent rise in cardiac output with sustained hypoxia.

Heart rate – acute responses

Our finding that propranolol caused a small reduction in G_{HR} is consistent with the observation by Petersen *et al*. (1974) that not all of the response of heart rate to acute hypoxia is mediated by the parasympathetic nervous system. In particular, it is consistent with their observation that β-blockade decreased the fall in heart rate following removal of a hypoxic stimulus.

Heart rate – subacute responses

We are unaware of any studies of the heart rate response to prolonged hypoxia over the kind of time period employed in the present experiment. Over longer periods of time, the changes in heart rate observed at altitude also appear to be unaffected by β-blockade (Mazzeo *et al*. 1994; Wolfel *et al*. 1994). However, increases in heart rate at altitude have been shown to be significantly correlated with increases in urinary noradrenaline (Mazzeo *et al*. 1998), and it therefore remains possible that a proportion of the increase in heart rate in response to prolonged hypoxia could occur by non-β-adrenergic sympathetic mechanisms.

From the above, it seems likely that sympathetic mechanisms play only a minor role in the hypoxic modulation of heart rate. Other alternative mechanisms that might underlie the progressive rise in heart rate with sustained hypoxia include the possibility that the rise in heart rate is effected by a direct action of hypoxia on the heart and that the rise is effected by a withdrawal of parasympathetic activity, as is the case for the majority of the response of heart rate to acute variations in the level of hypoxia (Petersen *et al*. 1974; Koller *et al*. 1988). If the mechanism does involve a withdrawal of parasympathetic tone, there are also questions relating to where the hypoxia is being sensed, and whether the effect arises directly or indirectly via changes in ventilation, for example acting via afferents from the pulmonary stretch receptors (see Daly, 1986 and Looga, 1997 for details). In relation to both the acute and the sub-acute responses of heart rate to hypoxia, the present study failed to demonstrate an indirect effect of ventilation in the sense that the correlations between the magnitude of acute ventilatory and heart rate responses to hypoxia failed to reach significance.

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