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

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Clar, Christine, Keith L. Dorrington, and Peter A. Robbins. Ventilatory effects of 8 h of isocapnic hypoxia with and without β-blockade in humans. J. Appl. Physiol. 86(6): 1897–1904, 1999.—This study investigated whether changing sympathetic activity, acting via β-receptors, might induce the progressive ventilatory changes observed in response to prolonged hypoxia. The responses of 10 human subjects to four 8-h protocols were compared: 1) isocapnic hypoxia (end-tidal PO₂ = 50 Torr) plus 80-mg doses of oral propranolol; 2) isocapnic hypoxia, as in protocol 1, with oral placebo; 3) air breathing with propranolol; and 4) air breathing with placebo. Exposures were conducted in a chamber designed to maintain end-tidal gases constant by computer control. Ventilation (Ve) was measured at regular intervals throughout. Additionally, the subjects’ ventilatory hypoxic sensitivity and their residual Ve during hyperoxia (5 min) were assessed at 0, 4, and 8 h by using a dynamic end-tidal forcing technique. β-Blockade did not significantly alter either the rise in Ve seen during 8 h of isocapnic hypoxia or the changes observed in the acute hypoxic ventilatory response and residual Ve in hypoxia about that period. The results do not provide evidence that changes in sympathetic activity acting via β-receptors play a role in the mediation of ventilatory changes observed during 8 h of isocapnic hypoxia.

METHODS

Subjects. We studied 10 subjects [6 men, 4 women; ages, 23.2 ± 3.3 (SD) yr; height, 181.3 ± 8.4 cm; weight, 72.1 ± 8.4 kg]. None of the subjects 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.

Protocols. The protocols were designed to allow us to compare the effects of hypoxia with and without β-blockade. Air-breathing protocols with and without the drug served as control exposures. Overall, the volunteers were subjected to four protocols on four different days (in varied order, with protocols separated by at least 1 wk). Female subjects were only studied during the first 2 wk of their menstrual cycles, unless they were taking a contraceptive pill, because levels of

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cycling progesterone are known to affect aspects of ventilation, such as CO₂ sensitivity (12, 13).

The conditioning associated with each protocol lasted 8 h. The four protocols were as follows. 1) Isocapnic hypoxia, in which PETO₂ was held at 50 Torr and PETCO₂ was maintained at the subject’s normal prehypoxic value. Four 80-mg doses of oral β-blocker (propranolol) were given every 8 h, starting 16 h before the experiment began (protocol IH-P). 2) Isocapnic hypoxia, as in protocol IH-P, except that, in this protocol, placebo tablets were given in place of propranolol at the same times as in protocol IH-P (protocol IH-C). 3) Air-breathing control, in which propranolol was given as in protocol IH-P (protocol C-P). 4) Air-breathing control, in which placebo was given as in protocol IH-C (protocol C-C).

VE was measured during these protocols at intervals of 0, 1, 2, 4, 6, and 8 h after the start. At 0, 4, and 8 h, the subjects’ AHVR and VE were determined under conditions of acute hypoxia. The PETO₂ profile used for these measurements is shown in Fig. 1. A 5-min lead-in period at a PETO₂ of 100 Torr was followed by six square waves, with PETO₂ alternating between 50 and 100 Torr and with each gas level being maintained for 1 min. After the last step, the PETO₂ was increased to 300 Torr and was maintained at that level for 5 min. PETCO₂ was kept at 1–2 Torr above the subject’s normal air-breathing value for the duration of these measurements.

Experimental technique. For the main 8-h hypoxic and control exposures, individual subjects were seated inside a clear-sided experimental chamber, where they could pursue activities such as watching television or reading. Inside this chamber, the ambient PO₂ and PCO₂ could be altered. The chamber and its control system have been described in more detail elsewhere (17). Isocapnic hypoxia, in which PETCO₂ was held at 50 Torr and PETCO₂ was maintained at the subject’s normal air-breathing value for the whole protocol. At the start of the experiment, the desired end-tidal gas values were entered manually into a controlling computer that regulated the gas composition inside the chamber. Every 5 min, the computer compared the average end-tidal gas values of the previous 3 min with the desired values, and the chamber gas composition was adjusted, if necessary, to keep end-tidal values constant. The chamber and its control system have been described in more detail elsewhere (17).

Measurements of VE in the chamber were undertaken by using a recursive inductance plethysmograph (Studley Data Systems, Oxford, UK). For each determination of VE, a 5-min measurement period was followed by a 5-min calibration period. During the measurement period, data were collected from the inductance plethysmograph with no disturbance of the subject. During the calibration period, subjects breathed via a mouthpiece and noseclip arrangement through a turbine volume-measurement device (SensorMedics VMM series, Cardiokinetics, Salford, UK) while data continued to be collected by using inductance plethysmography. The signals obtained from inductance plethysmography in the second period of 5 min could thus be calibrated by using the simultaneous measurements of respiratory volumes, and the calibration coefficients so obtained were then used to calibrate the data obtained in the first 5 min without the use of the mouthpiece and noseclip.

The responses to square waves of hypoxia to assess AHVR and to short periods of hypoxia were measured outside the chamber by using a mouthpiece and noseclip arrangement. Respiratory volumes were sensed with a turbine volume-measurement device. Respiratory flows and timing information were recorded with a Fleisch pneumotachograph. Gas was sampled continuously from a port close to the mouth, and inspired PO₂ and PCO₂ together with PETO₂ and PETCO₂ were measured by using a mass spectrometer (Airspec QP9000, Biggin Hill, UK). Before the start of the exposures, each subject’s normal PETCO₂ value was determined during a 5-min period of air breathing. During the exposure, the subjects also wore a pulse oximeter (Ohmeda Biox 3740, Louisville, KY) that served as a safety device.

Gas control was achieved by using a computer-controlled fast gas-mixing system (20). The required inspiratory gas composition was derived from a combination of a prediction of values from a model of the cardiorespiratory system and a breath-by-breath correction of the deviation of the actual values from the measured values. This prediction-correction scheme has been described in more detail elsewhere (32).

Model fitting. Numerical values for AHVR were obtained by fitting a model of the ventilatory response to acute hypoxia to the data obtained during the square waves of hypoxia. The particular model employed was model 3 of Clement and Robbins (9). In this model, total VE has been represented as the sum of ventilation at 100% saturation (VE₁), which has generally been ascribed to the central chemoreflex, and a ventilation which has been ascribed to the peripheral chemoreflex (VE₂). In the present experiments, PETCO₂ was maintained constant; therefore, VE₁ may be considered constant. Under conditions of both steady PETCO₂ and steady PETO₂, VE₂ would be equal to Gp(1 −S), where the gain term Gp is the hypoxic sensitivity at the fixed PETCO₂, representing the slope of the increase in VE with a decrease in saturation (5). Therefore, under these conditions, VE = VE₁ + Gp(1 −S).

However, under the conditions of dynamic hypoxic stimulation that we have been studying, it is also necessary to take into account the time constant (τ) that represents the time it takes to move toward a new steady-state value when the
saturation is changed, and the time delay ($t_d$) that represents the time it takes blood with a given saturation in the lungs to reach the carotid bodies, where the stimulus acts to produce the ventilatory response. The differential equation for this model is

$$\frac{dV_e}{dt} + \frac{V_e}{\tau} = \dot{V}_c + G_p [1 - S(t - t_d)]$$

By assuming that $S(t - t_d)$ remains constant from the beginning to the end of individual breaths, the equation may be solved to yield $V_e$ for breath $i$, as a function of the input ($S$), the parameters of the model, and the value of $V_e$ for breath $i-1$.

$$(V_e)_i = \left(\dot{V}_c + G_p [1 - S(t - t_d)]\right) - \left(\dot{V}_e\right)_{i-1} e^{-\tau(t-t_d)}$$

The parameters of this model are $\dot{V}_c$, $G_p$, $\tau$, and $t_d$. These parameters were estimated by nonlinear regression by using the Numerical Algorithms Group (Oxford, UK) Fortran library routine E04FD to minimize the sum of squares of the residuals. $S$ was calculated from the measured $PETO_2$ values by using the hemoglobin dissociation function as described by Severinghaus (35).

Statistical analysis. The main variables of interest were the values for $V_e$ in hypoxia or euoxia in the chamber (averages over the last 4 min of the 5-min measurement period were used), the values for $V_e$ in hyperoxia (averages over the last 3 min of the 5-min measurement period were used), and the values for $G_p$ and $V_c$ from the model fitting. ANOVA was used to test for significant differences between the responses to the four protocols. The particular factor of interest in the ANOVA was the interaction between drug, hypoxia, and time, because this addresses the question of whether the drug significantly altered, over time, the response of the respective variables to hypoxia.

RESULTS

All 10 subjects completed the study successfully, although some suffered from headaches during the second half of the hypoxic exposures.

Effectiveness of $\beta$-blockade. Table 1 shows the heart rate response at various time points for the four protocols. It can be seen that $\beta$-blockade of the heart was effective, as heart rate was always substantially lower than in the placebo protocols (ANOVA, $P < 0.001$).

Table 1. Heart rate measured in the chamber in each of the four protocols

<table>
<thead>
<tr>
<th>Time, h</th>
<th>Propranolol</th>
<th>Placebo</th>
<th>Propranolol</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>55 ± 2</td>
<td>71 ± 3</td>
<td>55 ± 2</td>
<td>66 ± 2</td>
</tr>
<tr>
<td>1</td>
<td>52 ± 2</td>
<td>66 ± 3</td>
<td>50 ± 1</td>
<td>58 ± 2</td>
</tr>
<tr>
<td>2</td>
<td>52 ± 3</td>
<td>70 ± 3</td>
<td>49 ± 2</td>
<td>58 ± 2</td>
</tr>
<tr>
<td>4</td>
<td>56 ± 4</td>
<td>71 ± 3</td>
<td>49 ± 2</td>
<td>59 ± 3</td>
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<td>83 ± 3</td>
<td>55 ± 1</td>
<td>66 ± 2</td>
</tr>
<tr>
<td>8</td>
<td>59 ± 2</td>
<td>77 ± 3</td>
<td>50 ± 2</td>
<td>61 ± 2</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, 10 subjects. Heart rate with propranolol significantly lower than with placebo; $P < 0.001$, ANOVA.

End-tidal gas values before and during protocols. Values for air-breathing $PETCO_2$ at the beginning of the experimental day were slightly but significantly ($P < 0.05$) lower in the presence of propranolol than when placebo had been taken (means ± SD of 5-min averages of breath-by-breath end-tidal values: propranolol protocols, 37.7 ± 4.5 Torr; placebo protocols, 39.1 ± 4.0 Torr). Figure 2, B and C, shows the gas control obtained in the chamber for the four protocols. Average values of $PETO_2$ (excluding $t = 0$) were 52.5 ± 0.8 Torr in protocol IH-P, 52.3 ± 0.6 Torr in protocol IH-C, 108.7 ± 5.9 Torr in protocol C-P, and 109.1 ± 3.1 Torr in protocol C-C. Average values of $PETCO_2$ were 37.5 ± 4.0 Torr ($-0.2 ± 0.8$ Torr difference from target value) in protocol IH-P, 38.9 ± 3.7 Torr ($-0.2 ± 0.3$ Torr difference from target value).
value) in protocol IH-C, 37.8 ± 4.8 Torr in protocol C-P, and 38.8 ± 3.3 Torr in protocol C-C.

\( V_E \) during hypoxia. Figure 2A shows the ventilatory response to the four protocols, as measured by inductance plethysmography in the chamber. Interestingly, no initial effect of hypoxia on \( V_E \) was apparent in these subjects, although this may simply be the result of rather high values for \( V_E \) at \( t = 0 \) in the chamber (compare \( t = 0 \) values with the remainder for the euoxic protocols). Nevertheless, \( V_E \) did increase with time during the hypoxic exposures (circles) but not during the air control experiments (squares). However, there was no significant difference between the response in the presence of propranolol (closed symbols) and in the presence of placebo (open symbols), during either the hypoxic exposure or the air control.

\( V_E \) during acute hyperoxia. Table 2 shows the mean \( V_E \) responses to the hyperoxic exposure during the last 3 min of the test. These values are somewhat higher than those observed under hypoxic conditions within the chamber. In part, this reflects the fact that the hyperoxic data were obtained at a somewhat higher PETCO2 than the hyperoxic data were, but it may also be related to the different techniques used to determine \( V_E \) under the two conditions (inductance plethysmography in hypoxia and turbine flowmeter with mouthpiece and noseclip in hyperoxia). It can be seen that in both hyperoxic protocols, \( V_E \) was elevated at 4 and 8 h (ANOVA, hypoxia by time, \( P < 0.001 \)). On inspection of the data, there appeared to be a difference in general baseline between the four protocols. This was confirmed by ANOVA (hypoxia by drug, \( P < 0.05 \)). However, there was no significant effect for the interaction between drug, hypoxia, and time, i.e., the drug did not affect the response over time to hypoxia.

\( V_E \) during square waves of hypoxia. Figure 3 shows a typical breath-by-breath individual response to the square waves of hypoxia used to assess AHVR and the subsequent period of hyperoxia. In this figure, a vigorous ventilatory response to hypoxia is shown, together with the good control over PETO2 and PETCO2 that was achieved by using the end-tidal forcing system. Figure 4 shows the average of the first five (in some cases 4) square waves averaged for all 10 subjects at \( t = 0, 4, \) and 8 h for the four protocols. It is evident from this figure that, in both hypoxic protocols, both \( V_E \) overall and the amplitude of the response to acute hypoxia were increased. Table 3 shows the corresponding values of Gp, Vc, τ, and τd obtained from the model for each protocol.

<table>
<thead>
<tr>
<th>Time, h</th>
<th>Propranolol</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( V_E ), l/min</td>
<td>PETCO2, Torr</td>
</tr>
<tr>
<td>0</td>
<td>17.4 ± 2.0</td>
<td>39.0 ± 1.2</td>
</tr>
<tr>
<td>4</td>
<td>27.0 ± 2.9*</td>
<td>39.2 ± 1.3</td>
</tr>
<tr>
<td>8</td>
<td>27.9 ± 3.1*</td>
<td>39.2 ± 1.2</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n = 10 \) subjects. Values for \( V_E \), PETCO2, and PETO2 are average values for last 3 min of the 5-min hyperoxic exposure.

*Significant rise in \( V_E \) over time with hypoxia compared with air control; \( P < 0.001 \), ANOVA.

Fig. 3. Example of measurements of hypoxic sensitivity and \( V_E \) during hyperoxia for 1 subject (breath-by-breath data). A: \( V_E \); B: PETO2; and C: PETCO2.
There was a significant increase over time in hypoxic sensitivity (G_p) in both hypoxic protocols (ANOVA, hypoxia by time, \( P < 0.001 \)). However, propranolol did not significantly affect the change in hypoxic sensitivity in response to hypoxia over time.

\( \bar{V} \dot{E} \) represents \( \bar{V} \dot{E} \) at 100% saturation. As such, it is notable that the calculated parameter from the model follows the same pattern as \( \bar{V} \dot{E} \) measured during hyperoxia, as described above. Just as in the case of the measured hyperventilation during hyperoxia, \( \bar{V} \dot{E} \) significantly increased over time during the hypoxic exposure (ANOVA, hypoxia by time, \( P < 0.001 \)), and there was a baseline difference between the protocols (ANOVA, hypoxia by drug, \( P < 0.05 \)). Again, however, the drug did not significantly affect the response of \( \bar{V} \dot{E} \) to hypoxia over time.

Sustained hypoxia significantly increased the time constant (\( \tau \)) which indicates the time taken for \( \bar{V} \dot{E} \) to reach a new steady-state level after the hypoxic step (ANOVA, hypoxia by time, \( P < 0.05 \)), but propranolol

Table 3. Parameters estimated for ventilatory model for acute response to hypoxia: for the 4 protocols at \( t = 0, 4, \) and 8 h

<table>
<thead>
<tr>
<th>Time, h</th>
<th>Propranolol</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G_p l·min(^{-1})·%(^{-1})</td>
<td>( \bar{V} \dot{E}), l/min</td>
</tr>
<tr>
<td>0</td>
<td>0.9 ± 0.1</td>
<td>18.9 ± 1.1</td>
</tr>
<tr>
<td>4</td>
<td>1.6 ± 0.3*</td>
<td>20.5 ± 1.5*</td>
</tr>
<tr>
<td>8</td>
<td>1.7 ± 0.3*</td>
<td>20.8 ± 2.4*</td>
</tr>
<tr>
<td>0</td>
<td>1.0 ± 0.2</td>
<td>12.8 ± 1.4</td>
</tr>
<tr>
<td>4</td>
<td>0.8 ± 0.1</td>
<td>11.7 ± 1.2</td>
</tr>
<tr>
<td>8</td>
<td>0.8 ± 0.2</td>
<td>11.4 ± 1.4</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n \), 10 subjects. G_p, gain term; \( \bar{V} \dot{E} \), calculated \( \bar{V} \dot{E} \) at 100% saturation; \( \tau \), time constant; \( t_d \), time delay. Significant rise in G_p, \( \bar{V} \dot{E} \), \( \tau \), and fall in \( t_d \) over time with hypoxia compared with air control; *P < 0.001, †P < 0.05, ANOVA. Significant increase in \( t_d \) with propranolol compared with placebo, \( P < 0.001 \), ANOVA.
had no significant effect on this parameter. Conversely, hypoxia significantly decreased the time delay (t.) (ANOVA, hypoxia by time, P < 0.05), and propranolol seemed to have the effect of increasing t. during both hypoxia and air control (ANOVA, P < 0.001) without abolishing the decrease seen during hypoxia.

DISCUSSION

The main finding of this study was that the early stages of the human ventilatory acclimatization to isocapnic hypoxia could not significantly be altered by β-adrenergic blockade with propranolol. In particular, the following features associated with an 8-h exposure to isocapnic hypoxia (PETO₂ = 50 Torr) were all unaffected by the drug: 1) the progressive increase in Ve, 2) the persistent hyperventilation observed during short spells of hyperoxia, and 3) the increase in AHVR observed during short periods in which PETO₂ was varied acutely.

Methodological considerations. Propranolol is a lipid-soluble, nonspecific, 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 that act at β₁- or β₂-receptors. The dose of propranolol used in this study was the same as that used in various studies of cardiorespiratory responses at altitude (1, 21, 26–28). Boden et al. (3) found that heart rate in humans was maximally blocked at an oral dose of propranolol of 200 mg/day, subdivided into doses given at 6-h intervals. This observation, coupled with the depression of heart rate that was observed in the present study (Table 1), suggests that the dose of propranolol employed was adequate for effective β-blockade.

Isocapnic vs. poikilocapnic hypoxia. The exposure to hypoxia that was employed in this study was isocapnic, and the question arises, To what extent are the findings likely to be applicable to hypoxic exposures in which the PETCO₂ is allowed to fall naturally, such as during travel to high altitude? As outlined in the introduction, the increases in both AHVR and residual Ve under conditions of acute hyperoxia have been found to be similar, whether or not the hypoxia exposure was maintained isocapnic (18, 36). Thus the influence (or lack thereof) of β-blockade is unlikely to be affected by the type of hypoxic exposure employed. More recent experiments have also found no difference between isocapnic and poikilocapnic exposures, even in the case of exposures lasting for 48 h (14, 37). These findings have also demonstrated that the increase in Ve under conditions of acute hyperoxia arises through an increase in ventilatory sensitivity to CO₂.

Comparison with other studies in humans. We are unaware of any studies directly comparable with the one presented in this paper, in which the focus has been on the early changes in the ventilatory acclimatization to isocapnic hypoxia and in which an attempt has been made to separate out different components of the respiratory response (AHVR, hyperoxic Ve) which may be mediated by different mechanisms. Two studies have investigated ventilatory changes with and without β-blockade during prolonged exposures to high altitude, i.e., during prolonged poikilocapnic hypoxia. Moore et al. (27) examined the ventilatory response of men to 15 days’ residence at Pikes Peak (4,300 m) with and without β-blockade (80 mg propranolol given every 8 h). Although there were differences in metabolic rate between the two groups, the progressive fall in PETCO₂ over time between the groups was similar; this suggests that β-blockade did not affect the process of ventilatory acclimatization to hypoxia. Asano et al. (1) also studied men on Pikes Peak, this time for 21 days with or without the same dose of propranolol, as used by Moore et al. In the study by Asano et al., Ve was not altered by the drug, but the ventilatory changes were related to urinary levels of norepinephrine. Therefore, these researchers suggested that there is a close link between ventilatory and sympathetic activation during hypoxia, but that this linkage is brought about by a non-β-adrenergic mechanism.

Although the finding is rather peripheral to the purposes of the present study, we were unable to detect any effect of β-blockade on the acute response to hypoxia at t = 0. This is in keeping with most other studies of the effect of β-blockade on the acute ventilatory response to hypoxia (4, 16, 22, 30). Only one study was able to demonstrate a significant depression of Ve with β-blockade (100 mg bupranolol), during both air breathing and hypoxia (8). The authors suggested that Ve increased less during hypoxia when the subjects received the β-blocker, although this effect appears to have been small.

Other possible mechanisms related to autonomic activity. There are various other ways in which the autonomic nervous system could modulate ventilatory control during hypoxia that are not dependent on β-adrenergic mechanisms. These include α-adrenergic mechanisms, central mechanisms independent of α- or β-receptors, and parasympathetic mechanisms.

In relation to α-adrenergic mechanisms, in the cat there is evidence that α₂-receptors are present in the carotid body and that they exert inhibitory influences (23). Furthermore, the inhibitory effect associated with intracarotid infusions of an α₂-receptor agonist on the carotid body appeared to be attenuated or lost after the animal had undergone a 24- to 36-h exposure to hypoxia before the infusion (6). This suggests that there may be a downregulation of the α₂-adrenergic inhibitory mechanism during the ventilatory acclimatization to hypoxia. However, other studies in other species were unable to confirm this finding (33). Moreover, O’Regan (29) was unable to abolish the increase in chemoreceptor discharge in response to sympathetic stimulation of the chemosensory cells with either an α- or a β-adrenergic blocker.

In relation to possible central mechanisms, adrenergic mechanisms are unlikely to play a role, because norepinephrine and epinephrine have been shown to exert a central depressant action on respiration (7).
activation stimulates neurons in the rostral ventrolateral medulla, and that these neurons, in turn, innervate and activate preganglionic sympathetic neurons of the spinal chord (31); this demonstrates that there are central links between the peripheral chemoreceptors and the sympathetic nervous system.

It is unlikely that slow changes in the parasympathetic input to the carotid body cause slow increases in ventilation during prolonged hypoxia, because parasympathetic efferents have an inhibitory influence on carotid body discharge (34, 38), and this inhibition may be increased after prolonged exposure to hypoxia (24).

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References


