Contribution of Pulmonary Receptors to the Heart Rate Response to Acute Hypoxemia in Rabbits

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We studied the effect of pulmonary afferent activity on the heart rate response to a progressive, isocapnic decrease in oxygen saturation (SaO₂) in anesthetized rabbits. To abolish the effect of rapidly adapting receptors, we used inhaled bupivacaine aerosol, and to abolish the effect of slowly adapting stretch receptor activity, we used sulfur dioxide insufflation. The heart rate (HR) response (ΔHR/ΔSaO₂) under control conditions was 0.39 ± 1.29 beats/min/% (mean ± SD, n = 11; values >0 indicate a tachycardiac response to hypoxemia). After sulfur dioxide insufflation, all nine rabbits had a bradycardiac response (−2.02 ± 1.13 beats/min/%), which was significantly less than control (p<0.0001). After bupivacaine inhalation, the heart rate response (0.27 ± 1.04 beats/min/%) was unchanged from control. There were no significant differences in the percent increase of minute ventilation during hypoxemia in all runs. Our results indicate that in rabbits the receptors responsible for the increase in heart rate during progressive hypoxemia are the slowly adapting receptors. (Circulation 1988;78:1260–1266)

Heart rate response to acute hypoxemia is mediated by complex neural interactions between peripheral chemoreceptors and pulmonary reflexes. Previous studies by Daly and Scott, as well as studies from our group, have suggested that slowly adapting stretch receptors play an important role in producing the tachycardiac response to hypoxemia in dogs. To date, however, the data supporting this hypothesis have been largely circumstantial, and it is possible that other receptors, such as the rapidly adapting receptors (cf., irritant receptors) contribute to the heart rate response.

Davies and colleagues recently developed a method of sulfur dioxide insufflation whereby selective blockade of slowly adapting receptors can be achieved in rabbits while rapidly adapting receptors and J-receptor activities remain intact. In the present study, we used this model of selective blockade of slowly adapting receptors as well as airway anesthetia to block the cough reflex to examine which type of pulmonary receptor mediates the heart rate response to acute hypoxemia.

Materials and Methods

Experimental Preparation

Eleven New Zealand White rabbits of either sex (body weight, 3.0–4.5 kg; mean ± SD, 3.84 ± 0.44 kg) were anesthetized with a mixture of ketamine hydrochloride (100 mg/ml) and xylazine hydrochloride (20 mg/ml) (loading dose, 0.5 ml/kg i.m.; supplemental dose, 0.05–0.15 ml/kg/30 min i.v.). A catheter was placed into an ear vein for administration of fluids and drugs. Femoral artery catheters were inserted to sample blood for determination of blood gases (Model 168, Corning, Corning, New York) and to measure blood pressure (PD23, Gould-Statham, Cleveland, Ohio). Beat-by-beat heart rate was obtained from the pulse interval of the pressure waveform with a Gould Biotach amplifier. An endotracheal tube was inserted by a tracheostomy; a T-piece at the airway opening was connected to a circuit through which a gas mixture of oxygen, nitrogen, and carbon dioxide were delivered (Matheson flow controller, Secaucus, New Jersey). Esophageal pressure (MP 45-30, Validyne Engineering, Northridge, California) was measured with an esophageal balloon. A pneumotachygraph (Fleish #1) was inserted between the endotracheal tube and T-piece to measure the tidal volume by integration of the flow signal. Oxygen saturation (SaO₂) was monitored with a Biox II oximeter placed on the tongue of the rabbit. Core temperature (Yellow Springs Instruments, Yellow Springs, Ohio) was maintained at 39.0 ± 1.0°C with a heated surgical
table. Normal acid-base balance was maintained throughout the experiment by administration of NaHCO₃ as required. Fluid status was maintained with normal saline, infused continuously at 5 ml/kg/hr with a Harvard pump (South Natick, Massachusetts).

**Experimental Protocols**

The strength of the Hering-Breuer inflation reflex was assessed as the apnea duration induced by the inflation of the lungs with oxygen at end inspiration, to a pressure of 10 cm H₂O; in two rabbits, we used 5 cm H₂O. This method is similar to that used by Davies and colleagues.⁷ As an index of the strength of the Hering-Breuer inflation reflex, we used the ratio (dT) of the apnea duration to the averaged expiratory duration of the five preceding breaths under normocapnic, hyperoxic conditions. The cough reflex was elicited by inserting a fine polyethylene tube into the trachea as far as the tracheal bifurcation. These pulmonary reflexes were examined before and after every hypoxic run.

After stabilizing the rabbit under normocapnic, hyperoxic conditions, progressive hypoxemia was induced during 8–10 minutes by continuously decreasing the oxygen flow and increasing the nitrogen flow until an SaO₂ of approximately 60% was attained. If the rabbit was not able to tolerate such low levels of SaO₂, we terminated the run at SaO₂ greater than 60%. During the run, expired gas was sampled to measure end-tidal carbon dioxide concentration (Sensor-Medics LB2, Anaheim, California), and it was maintained constant by adding carbon dioxide to the inspired gas. Arterial blood gases were drawn anaerobically after every 5% change in SaO₂. The blood gas measurements were used to ensure a constant PaCO₂ during the course of hypoxic induction and were also used to correct SaO₂ after the experiment (see below). Results of experiments in which the PaCO₂ varied by more than ±2 mm Hg within a run were discarded.

After the control hypoxic runs, in six rabbits, we used the technique of Jain and colleagues⁸ with inhaled bupivacaine to abolish the cough reflex selectively. Bupivacaine hydrochloride was dissolved in saline to make a 5% solution. The aerosol was generated in a Wright nebulizer (British Oxygen, London, UK) with heated oxygen at a constant flow rate of 15 l/min. After removal of the pneumotachygraph, the aerosol from the nebulizer was administered to the lungs through the endotracheal tube by clamping the expiratory portion of the circuit. As soon as the esophageal pressure rose to 10–20 cm H₂O, the lungs were kept inflated for approximately 10 seconds by clamping the endotracheal tube side of the T-piece. The cough reflex was tested, and the inhalation of bupivacaine aerosol was repeated until the cough reflex was abolished. With this method, blocking the cough reflex was possible with a smaller dose of bupivacaine aerosol than that required to abolish the Hering-Breuer inflation reflex. After assessing the Hering-Breuer reflex, progressive hypoxemia was induced as described above.

The effect of insufflated sulfur dioxide on the heart rate response was examined in nine rabbits; four of these had also been studied after bupivacaine inhalations. By the technique of Davies and colleagues, sulfur dioxide (Union Carbide, Toronto, Canada) was given in a concentration of 200 ppm in air for 10–15 minutes by continuous insufflation into the trachea at a constant flow of 10 l/min until the Hering-Breuer inflation reflex was abolished. Progressive hypoxemia was then induced in the manner described above.

**Data Analysis**

Although SaO₂ was measured continuously with the Biox II oximeter, on a number of occasions, we observed a discrepancy between the oximeter measurements and those expected on the basis of measured arterial blood gases. Accordingly, we calculated SaO₂ from the blood gas measurements with an equation derived from the oxygen dissociation curves of rabbits.¹¹ The equation was valid only throughout the range of SaO₂ between 60% and 95%, and hence, only data obtained throughout this range were used for analysis.

The data, recorded on a Gould ES1000 recorder, were then analyzed with a digitizer (Super-Grid, Summagraphics, Fairfield, Connecticut) connected to a computer (IBM-PCXT). The heart rate was measured for 0.5–1.0% decrements in SaO₂, and when there was respiratory sinus arrhythmia, the heart rate was averaged over the duration of each breath. The values of tidal volume and respiratory frequency at the same level of SaO₂ were also calculated.

The data were then averaged at 5% SaO₂ intervals, and the results were plotted as the heart rate versus SaO₂. The response was described as the slopes (ΔHR/ΔSaO₂) by linear regression analysis.¹² All values are presented as mean ± SD. Two-tailed paired t tests were used to evaluate the significance of the difference between paired sets of data.

**Results**

There were no significant differences in the mean values for PaCO₂, baseline heart rate, baseline mean arterial blood pressure, and the increase in mean arterial blood pressure during the hypoxic run among study groups (Table). Baseline values were obtained by averaging data throughout the range of SaO₂ from 95% to 90%. We calculated the percent increase in minute ventilation during hypoxia, which is the quotient of the increase in minute ventilation divided by the baseline minute ventilation. There was no significant difference in the percent increase in minute ventilation among the different experimental conditions.

**Effect of Sulfur Dioxide Insufflation on Heart Rate Response to Hypoxemia**

Baseline values of dT ranged from 2.2 to 23.0 (11.6 ± 5.7) under control conditions (Figure 1). Sulfur
dioxide insufflation caused a significant decrease in dT to a mean value of 1.6, indicating practical abolition of the Hering-Breuer inflation reflex (Figure 1). However, we could not observe the cough reflex after sulfur dioxide insufflation in five rabbits, although it was present before sulfur dioxide insufflation.

Arterial blood pressure, esophageal pressure, tidal volume, and heart rate under control conditions and after sulfur dioxide exposure are presented in Figure 2. In this rabbit, the heart rate increased as Sao₂ decreased during the control hypoxic run, whereas hypoxemia after sulfur dioxide exposure caused the heart rate to decrease. Figure 3 presents the heart rate versus Sao₂ data from a rabbit whose response to hypoxemia was qualitatively representative of the majority of the rabbits studied, with an increase in heart rate under control and postbupivacaine conditions and a decrease in heart rate after sulfur dioxide exposure. Individual results for all of the experiments are summarized in Figure 4, with the negative of the slope (∆HR/∆Sao₂) of the heart rate versus Sao₂ plots. Values greater than zero indicate increased heart rate as Sao₂ decreased. As shown in Figure 4, under control conditions, the heart rate response was varied (increase, decrease, or no change in the heart rate), whereas after sulfur dioxide exposure, the response was always a bradycardiac one, which was significantly decreased compared with the control studies (p<0.0001, n=9).

**Effect of Bupivacaine Inhalation on Heart Rate Response to Hypoxemia**

Bupivacaine inhalation abolished the cough reflex with no significant effect on dT (control, 13.5±5.4 vs. bupivacaine, 15.6±8.4; p=0.27, n=6) (Figure 1). An experimental recording of heart rate, arterial blood pressure, esophageal pressure, and tidal volume for one rabbit are presented in Figure 5. After bupivacaine inhalation, this rabbit exhibited roughly the same tachycardiac response to hypoxemia as observed during the control run. As shown in Figures 3 and 4 and in the Table, there was no significant difference in ∆HR/∆Sao₂ between control and bupivacaine runs (p=0.90, n=6). In all four rabbits in whom both interventions (sulfur dioxide insufflation and bupivacaine inhalation) were used, ∆HR/∆Sao₂ after sulfur dioxide inhalation was significantly decreased (p<0.05, n=4) compared with that after bupivacaine inhalation (Figure 4).

Figure 6 depicts the relation between heart rate response (∆HR/∆Sao₂) and dT during control runs in nine rabbits in whom inflations to 10 cm H₂O were used to examine dT. With linear regression analysis this relation was significant (r=0.69, p<0.05, n=9).

**Discussion**

In the present study, we examined the effect of pulmonary receptors on the heart rate response to isocapnic progressive hypoxemia in anesthetized, spontaneously breathing rabbits. Our main findings are that 1) isocapnic progressive hypoxemia produced a varied heart rate response: increase, decrease, or no change in heart rate during control hypoxic runs; 2) the variability of heart rate response during control runs was correlated with dT, the strength of Hering-
Breuer inflation reflex; 3) after abolition of the cough reflex, the heart rate response was not significantly different from those obtained during the control runs; and 4) the abolition of the Hering-Breuer inflation reflex always caused a bradycardiac response and significantly decreased the heart rate response as compared with that during control runs.

Our studies used an anesthetized preparation that could theoretically affect vagal reflexes. Gupta and Singh investigated the effects of anesthetic agents on the heart rate response to hypoxemia and concluded that the resultant response was dependent on the prevalent autonomic drive; dogs with high resting heart rates exhibited a bradycardiac response, whereas dogs with relatively low resting heart rates exhibited a tachycardiac response. The choice of anesthetic agent resulted in baseline heart rates in our experiments that were comparable to the rest-
ing heart rates measured in unanesthetized rabbits and, hence, would reflect normal cardiac vagal tone. Furthermore, the baseline heart rates of our three groups were practically the same (Table), yet the heart rate responses were markedly different (Figure 2). Hence, it is unlikely that our observations are due to an anesthetic effect.

Our results appear to be different than those of Korner and colleagues, who observed a bradycardiac response to hypoxemia, with most rabbits exhibiting a tachycardiac response and the rest displaying either a bradycardiac response or no change in heart rate. This discrepancy is probably related to the intensity of the hypoxic stimulus that we used. We examined the heart rate response down to an SaO₂ of about 60%, which is equivalent to a PaO₂ of approximately 35 mm Hg in rabbits. A PaO₂ of this level was considered moderate hypoxia in Korner’s study, and he demonstrated that this degree of hypoxia resulted in a tachycardiac response and that further reductions of PaO₂ were associated with a bradycardiac response in unanesthetized rabbits.

To identify the specific receptors responsible for the tachycardiac response, we used two techniques of abolishing receptor activity. We used sulfur dioxide insufflation in an attempt to block slowly adapting receptors in a selective fashion. We were not entirely successful in this regard because after sulfur dioxide insufflation we could not detect a cough reflex in some of our rabbits, although the rabbits coughed on the first introduction of sulfur dioxide into the lungs. One explanation for this attenuation of the cough reflex could be habituation of rapidly adapting receptor activity to the constant irritant stimulus of sulfur dioxide. Nevertheless, the data from these experiments clearly indicated that the receptors that were inhibited by sulfur dioxide were responsible for the tachycardiac response (Figures 3 and 4). Because sulfur dioxide exposure affected both rapidly adapting and slowly adapting receptor activity, we were uncertain which receptor was the critical one in generating the tachycardiac response; hence, we used bupivacaine inhalation. Jain and colleagues have shown that bupivacaine inhalation does not block J-receptors but can block both the Hering-Breuer inflation reflex or the cough reflex or both, depending on the dose of bupivacaine aerosol. We attempted to take advantage of this dose-dependent response by administering small doses of bupivacaine aerosol, sufficient to block the cough reflex but not slowly adapting receptors. We were successful in this regard in that the cough reflex was abolished, but the Hering-Breuer inflation reflex was not affected (Figure 1). After bupivacaine inhalation, the heart rate response to hypoxemia was unaffected. Thus, taken together, these two models provide very strong evidence that the receptors responsible for the tachycardiac response to hypoxemia are the slowly adapting receptors (pulmonary stretch receptors).

The variability in heart rate response observed in the present study is similar to that observed in our previous study in anesthetized dogs. Further, we observed a significant relation between ΔHR/ΔSaO₂ and the strength of Hering-Breuer inflation reflex (Figure 6). These data support the conclusion that the tachycardiac response to hypoxemia is mediated by slowly adapting receptor (pulmonary stretch receptors) afferents.

In addition, other receptors such as J-receptors, which can reflexly decrease heart rate after chemical stimulation, have to be considered as a possible contributing mechanism. Paintal suggested that J-receptors (pulmonary C-fibers) can be stimulated by the increase in mean pulmonary artery pressure that often accompanies exercise and that this...
stimulus might provide the afferent input that limits exercise. Because it is possible that pulmonary artery pressures were increased during the induction of hypoxia, stimulation of these receptors is a possible explanation for our results. We think that this is unlikely because both methods that we used to eliminate receptor afferent activity are thought not to affect J-receptors.7,8 Another possibility, that we have effectively ruled out, is the contribution of afferent information from respiratory muscles on the heart rate response because it is unlikely that sulfur dioxide exposure directly alters this afferent loop. Further, there is unlikely to be an indirect effect because the change in minute ventilation was not different for the control and sulfur dioxide-exposure studies.

Before extrapolating the conclusions of this study to humans, it is important to address the issue of whether the Hering-Breuer reflex exists in humans. Widdicombe21 found that in humans lung inflation caused apnea (through the Hering-Breuer reflex) but that it was of a shorter duration than that
observed in other species. Although the Hering-Breuer reflex was weak with inflation volumes comparable to the tidal volume, inflation volumes of 1,000 ml or more produced marked respiratory inhibition, indicating that the Hering-Breuer reflex is activated at higher lung volumes when compared with other species. Hence, although this reflex may not play a role during normal tidal breathing it is likely to be recruited under conditions of increased ventilation such as occurs during hypoxia.

There are also data that suggest that the heart rate response to hypoxia in humans is similar to that of other animals such as dogs or rabbits. Premature infants usually become bradycardiac during hypoxic apneic episodes. Similarly, the periods of upper airway obstruction that occur in patients with obstructive sleep apnea are often associated with a marked bradycardia, which becomes a tachycardia, at the onset of ventilation, even though the oxygen saturation may be slightly lower. Phylogenetically, the bradycardiac response may be a vestige of a primitive diving reflex.

In conclusion, our results strongly suggest that, in rabbits, the receptors responsible for the increase in heart rate response to progressive hypoxia are the slowly adapting stretch receptors.

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References

1. Daly MD, Scott MJ: The effects of stimulation of the carotid body chemoreceptors on heart rate in the dog. J Physiol (Lond) 1958;144:148–166
17. Coleridge HM, Coleridge JCG, Luck IC: Pulmonary afferents fibers of small diameter stimulated by capsaicin and by hyperinflation of the lung. J Physiol (Lond) 1965;179:248–262

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Figure 6. Plot of relation between dT and heart rate response. dT is the index of the strength of Hering-Breuer inflation reflex (see text). Measurements of dT were obtained with inflations to 10 cm H2O. Correlation coefficient is 0.69 (p<0.05, n=9).
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