Peripheral Chemosensitivity Assessed by the Modified Transient O₂ Test in Female Twins*

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To evaluate genetic influence on the control of breathing in adult women, we measured, in healthy female twins, ventilatory responses to isocapnic progressive hypoxia and hyperoxic progressive hypercapnia, and the withdrawal response (the modified transient O₂ test) which is considered to selectively reflect peripheral chemoreceptor activity. The withdrawal response was obtained as the magnitude of initial depression in ventilation induced by two breaths of O₂ from steady-state hypercapnic hypoxia. Nine monozygotic twin pairs, aged 44±SD17 years, and 7 dizygotic twin pairs, aged 39±5 years, were studied. Mean values for ventilatory responses to hypoxia and hypercapnia, and the withdrawal response were not different between MZ and DZ. The within-pair variance ratio (Vdz/Vmz) for the withdrawal response was significantly greater than one (p<0.05), although neither Vdz/Vmz for the hypoxic response nor that for the hypercapnic response was greater than one. These observations suggest that the peripheral chemosensitivity is influenced by genetic factors even in adult women, including aged subjects, when genetic influence is not apparent in the ventilatory responses to progressive hypoxia and hypercapnia.

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Vertilatory response to isocapnic progressive hypoxia reflects not only peripheral chemosensitivity, but also the central component of ventilatory control, which includes central hypoxic depression. The withdrawal response (modified transient O₂ test) is a transient ventilatory change induced by sudden elimination of hypoxia and is considered to more selectively measure the peripheral chemoreceptor activity than the ventilatory response to progressive hypoxia. In the present study of healthy adult female twins, we thus examined the withdrawal response, as well as ventilatory responses to isocapnic progressive hypoxia and hyperoxic progressive hypercapnia, to clarify the predominant site of genetic influence on the control of breathing.

MATERIALS AND METHODS

Subjects

Sixteen pairs of adult female twins agreed to participate in the study. They were all healthy at the time of the study. None of the twins was a regular athlete or long-distance swimmer. Informed consent was obtained from all participants, and the study was approved by the Ethics Committee.

The subjects included nine pairs of monozygotic and seven pairs of dizygotic twins. The ages, physical characteristics, smoking habits, and respiratory functions are listed in Table I. The twins were classified into MZ and DZ by blood groups (ABO, Rh, MN, Kell, S, Lewis, Duffy, Kidd, Lutheran, P, and Xg), fingerprints, and physical characteristics; MZ was defined when all the blood groups were identical, more than seven fingerprints matched, and physical characteristics such as eyebrows, noses, and ear lobe attachments were very similar.

The MZ twins had lived apart for 27±SD18 yr (range, 1 to 44 yr) and the DZ twins for 21 ± 13 yr (range, 6 to 49 yr). Three of the 18 MZ and one of the 14 DZ twins were current smokers. There were also three exsmokers in MZ and one in DZ twins. Eleven MZ and 11 DZ twins were before menopause. Co-twins in each pair were examined on the same day to ensure reliability in zygosity determination by blood typing; therefore, menstrual phases on the examination day were not always the same in each pair. The coincidence ratios for the menstrual cycle, assessed by dates of the

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Test for Peripheral Chemosensitivity (Akiyama et al)
last menstruation and mean menstrual cycles, were not different between MZ and DZ.

**Ventilatory Response Tests**

For all the measurements of ventilatory responses, each subject was in the supine position with her eyes closed and breathed spontaneously through a mouthpiece connected to a J valve. A dual-control system was used to regulate arterial PaO, (PaO2) and PCO2 (PaCO2) simultaneously and independently. Briefly, the system utilized end-tidal PaO2 (PtO2) and PCO2 (PtCO2) as guides to control arterial blood gases by automatically changing inspiratory gas composition. Minute ventilation (Ve) was measured every 15 s by electrical integration of the flow signal obtained from a hot-wire respiratory flow meter. Respiratory gases were continuously monitored by a mass spectrometer. The signals of Ve, PtO2, and PtCO2 were continuously monitored and recorded on a multichannel recorder, and at the same time, stored at 15-s intervals in an on-line signal processing computer for later analysis.

Arterial blood samples were drawn from the brachial artery while the subject was breathing room air through the mouthpiece. Blood gases and pH were analyzed soon after sampling with a polarographic technique (pH/blood gas analyzer). Ventilatory responses to isocapnic progressive hypoxia, hyperoxic progressive hypercapnia, and the withdrawal response were measured in this order with at least 30-min intervals. Isocapnic hypoxia was attained by progressively lowering PaO2 from 100 to 40 mm Hg over 6 to 7 min. The ventilatory response to isocapnic progressive hypoxia was assessed by the slope factor (A) for the ventilation-PaO2 curve,

\[ \text{Ve} = \text{Vo} + A(\text{PaO}_2 - 30) \]

where Ve is minute ventilation in L/min (BTPS), Vo is the asymptote for ventilation obtained by extrapolation; A is the slope factor of the Ve-PaO2 curve in L/min/mm Hg *mmHg-1; and 30 is the asymptote for PaO2 in mm Hg when Ve is infinite.

For hyperoxic hypercapnia, the initial inspired fraction of CO2 (3 percent) was set to allow the predetetermined PtCO2 (80 mm Hg) over 5 to 6 min while PtO2 was maintained at 180 mm Hg. Hypercapnic ventilatory response was evaluated by the slope for the ventilation-PtCO2 line using the following equation:

\[ \text{Ve} = S(\text{PtCO}_2 - B) \]

where S is the slope of the Ve-PtCO2 line in L/min/mm Hg *mmHg-1 and B is the intercept of the Ve-PtCO2 line with PtCO2 in mm Hg.

Peripheral chemoreceptor activity was evaluated by the transient O2 test advocated by Miller and colleagues and modified later by Honda and co-workers. Briefly, this test measures a fall in ventilation following sudden elimination of hypoxia. Inspired gas composition was adjusted to obtain a PaO2 of 55 mm Hg and a PtCO2 of 5 mm Hg, higher than the resting value using a dual-control system of arterial blood gases. These end-tidal gas values were maintained for at least 3 min after the ventilation became stable. Then, two breaths of 100 percent oxygen were administered, without the subject being aware, by turning a three-way stopcock near the inlet of the J valve connected to the mouthpiece. Mean Ve was calculated for all breaths from 5 to 20 s after the inhalation of pure oxygen and was subtracted from the mean Ve during the previous 3 min. This difference was defined as ΔVe, the withdrawal response from hypoxia. Withdrawal response was measured four times in each subject, and the averaged values of four measurements were used for evaluation.

A, S, and ΔVe were standardized by body surface area (BSA), a known determinant of ventilatory response.

**Statistics**

Basically, statistical methods for twin studies were used. Because the number of subjects was relatively small in the present study, a method of comparing the within-pair variance without regard to whether total variances between MZ and DZ were equal was selected. Whether the variable of interest was more similar between MZ pairs than between DZ pairs was assessed by the within-pair variance ratio between MZ and DZ, the within-pair variance of MZ being the denominator. When the ratio was significantly larger than one, the similarity between MZ pairs was thought to be stronger than that between DZ pairs. Because the data were always tested for a unidirectional change, a single-tailed F test was used for this purpose. Differences between the two group means were examined by Student's t-test. The p values of less than 0.05 were accepted as significant.

**RESULTS**

Mean values for age, physical characteristics, years of separation, smoking index, and pulmonary function tests were not different between the MZ and DZ groups (Table 1). There were no differences between MZ and DZ in mean values for arterial blood gases, A/BSA, S/BSA, and ΔVe/BSA (Table 2). Within-pair variance ratios (F values) for physical characteristics and ventilatory response values are shown in Table 3. Within-pair variance ratios for body height, body weight, body surface area, and ΔVe/BSA were significantly greater than one, indicating that these variables are influenced by genetic factors. Within-pair variances for A/BSA, and S/BSA were not different between MZ and DZ. A comparison between ΔVe/BSA values of the two groups is shown in Figure 1.

**DISCUSSION**

Several twin studies have examined genetic influences on chemical control of breathing. Arkinstall and

**Table 1—Comparison Between Monzygotic and Dizygotic Twins**

<table>
<thead>
<tr>
<th></th>
<th>MZ</th>
<th>DZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pairs, No.</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>Age, yr</td>
<td>44±17</td>
<td>39±8</td>
</tr>
<tr>
<td>Height, cm</td>
<td>152.4±5.0</td>
<td>149.9±5.5</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>53.6±7.7</td>
<td>52.1±9.0</td>
</tr>
<tr>
<td>BSA, m²</td>
<td>1.49±0.10</td>
<td>1.45±0.13</td>
</tr>
<tr>
<td>Separation, yr</td>
<td>27±18</td>
<td>21±13</td>
</tr>
<tr>
<td>Smoking, pack-years</td>
<td>2.5±5.8</td>
<td>0.5±1.2</td>
</tr>
<tr>
<td>VC, L</td>
<td>2.83±0.53</td>
<td>2.81±0.45</td>
</tr>
<tr>
<td>FEV₁, L</td>
<td>2.38±0.70</td>
<td>2.53±0.40</td>
</tr>
<tr>
<td>FEV₁/FVC, %</td>
<td>82±12</td>
<td>85±4</td>
</tr>
</tbody>
</table>

*Results are shown as means±SD. The p value was not significant in any category.

**Table 2—Data for Chemical Control of Breathing**

<table>
<thead>
<tr>
<th></th>
<th>MZ</th>
<th>DZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.384±0.034</td>
<td>7.388±0.024</td>
</tr>
<tr>
<td>PaCO₂, mm Hg</td>
<td>38.4±3.9</td>
<td>38.4±2.8</td>
</tr>
<tr>
<td>PaO₂, mm Hg</td>
<td>98.8±17.2</td>
<td>97.6±10.4</td>
</tr>
<tr>
<td>HCO₃⁻, mEq/L</td>
<td>23.2±2.9</td>
<td>24.0±1.9</td>
</tr>
<tr>
<td>A/BSA, L/min⁻¹·mm Hg⁻¹·m⁻³</td>
<td>57.2±38.6</td>
<td>54.5±45.9</td>
</tr>
<tr>
<td>S/BSA, L/min⁻¹·mm Hg⁻¹·m⁻³</td>
<td>0.78±0.47</td>
<td>0.92±0.45</td>
</tr>
<tr>
<td>ΔVe/BSA, L/min⁻¹·m⁻³</td>
<td>3.0±2.7</td>
<td>4.1±2.3</td>
</tr>
</tbody>
</table>

*Results are shown as means±SD. The p value was not significant in any category.
co-workers\(^1\) reported that the response of tidal volume to hypercapnia was more similar within pairs of MZ than within DZ pairs. Collins and colleagues\(^2\) showed that the hypoxic ventilatory response is genetically determined. Kawakami and co-workers\(^3\) reported that respiratory chemosensitivity to both hypoxia and hypercapnia is controlled by genetic factors in adults as well as in adolescents,\(^4\) although age-related variations were seen even in MZ twins.\(^5\)

In the present study, using adult female twins alone as subjects, we have demonstrated genetic influence on peripheral chemosensitivity assessed by the modified transient \(O_2\) test, although genetic influence was not apparent in the ventilatory responses to progressive hypoxia and hypercapnia. There could be several explanations for the negative results concerning genetic factors in the ventilatory responses to progressive hypoxia and hypercapnia. First, the mean age of the subjects was higher and the mean period of separation was longer in the present study than in previous twin studies.\(^6,7\) Within-pair variations in environmental factors after twins started to live separately might become large enough, in a time-dependent fashion, to mask genetic influences on inherent chemosensitivity. Possible environmental factors include changes in physical characteristics, pulmonary mechanics, metabolic rate,\(^8\) acid-base status,\(^9\) smoking habits,\(^10\) athletic activities,\(^11\) residence at high altitude,\(^12\) and others. Dietary habits, as well as smoking and athletic activities, may affect arteriosclerosis, and consequently, brain blood flow, which influences hypoxic ventilatory responses.\(^13\) This study suggests that such environmental factors mainly affect integration or processing in the respiratory center and/or central chemoreception rather than peripheral chemoreceptor activity. Second, there might be within-pair variations in female gonadal hormones because menstrual phases (follicular phase, luteal phase or postmenopausal pe-

\[
\Delta \dot{V}E/BSA, \text{ L } \cdot \text{min}^{-1} \cdot \text{m}^{-2}
\]

![Figure 1](image)

\[F = V_{DZ}/V_{MZ} = 4.81 \quad (p < 0.05)\]

**Figure 1.** The withdrawal responses of co-twins in MZ (left panel) and in DZ (right panel). The within-pair variance ratio, in which the variance in MZ was the denominator, was significantly larger than one \((p<0.05)\).
roid) on the examination day were not always the same in each pair. Among such hormones, progesterone has been reported to increase ventilatory responses to hypoxia and hypercapnia.\textsuperscript{7,8,10} Because these positive effects of progesterone on respiratory chemosensitvity have been suggested to derive from central rather than peripheral action,\textsuperscript{22} the alterations in control of breathing due to this hormone might be detectable only by the overall ventilatory responses to hypoxia and hypercapnia. Considering the relatively low reproducibility of the ventilatory response tests,\textsuperscript{83} the negative results with respect to genetic effects on hypoxic and hypercapnic responses might be simply due to the small number of subjects examined in the present study.

We have recently demonstrated that genetic influence is not seen in behavioral aspects of the control of breathing\textsuperscript{84} and that such behavioral control may affect ventilatory responses in conscious humans.\textsuperscript{85} There have been several reports showing that behavioral style or personality has some influence over the ventilatory responses.\textsuperscript{86,87} Therefore, variations in influences from the higher brain may also explain the present findings.

Nevertheless, we have demonstrated genetic influence on peripheral chemoreceptor activity even in adult women, including aged subjects. This suggests that the peripheral chemosensitivity is strongly determined by genetic factors, irrespective of age and gender.

REFERENCES

1. Arkinstall WW, Nirmel K, Kissouras V, Mille-Emili J. Genetic differences in the ventilatory response to inhaled CO\textsubscript{2}. J Appl Physiol 1974; 36:6-11