Dextromethorphan affects ventilation differently in male and female rats

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Schlenker, Evelyn H. Dextromethorphan affects ventilation differently in male and female rats. J. Appl. Physiol. 81(5): 1911–1916, 1996.—Subcutaneous administration of aspartic acid results in a long-lasting but reversible depression of ventilation in male but not in female rats. Aspartic acid acts on N-methyl-D-aspartate receptors. The present study tested the hypothesis that a noncompetitive N-methyl-D-aspartate-receptor antagonist, dextromethorphan (Dex), would depress ventilation in female rats and stimulate it in male rats. Moreover, Dex administered prior to aspartic acid should prevent the aspartic acid-induced depression of ventilation in male rats. In female rats, Dex caused a 30% depression of ventilation relative to saline at 5 and 10 mg/kg (P < 0.01) but not at the highest dose (20 mg/kg). In male rats, Dex had no effect on ventilation. At a dose of 20 mg/kg, Dex depressed oxygen consumption to 50% of the saline value at all time points in female rats (P < 0.001) and in male rats 45 and 60 min after administration. The time points when Dex depressed ventilation and oxygen consumption were different in female rats, suggesting that the depression of ventilation was not the result of a depression in oxygen consumption. During a hypercapnic challenge (7% CO₂), female rats treated with 5 and 10 mg/kg of Dex exhibited a smaller increase in ventilatory response relative to saline treatment. At a dose of 20 mg/kg, the hypercapnic responsiveness of male rats was markedly stimulated (85.8 ± 8.95 ml/min) relative to saline (50.6 ± 9.14 ml/min; P < 0.001). Finally, Dex administered before aspartic acid prevented the aspartic acid-induced depression of ventilation in male rats. Thus, in rats, Dex has gender-specific effects on ventilation and these effects are not associated with changes in oxygen consumption.

With this background, the present study was designed to test the hypothesis that dextromethorphan (Dex), a noncompetitive NMDA-receptor antagonist (7, 8, 26), would cause a depression in breathing in female rats but not in male rats. Furthermore, Dex administered before aspartic acid should prevent the aspartic acid-induced depression of ventilation in male rats. This antagonist was selected because, unlike MK-801 (dizocilpine), Dex does not have the psychotomimetic effects (10, 28). Subcutaneously administered Dex also readily and rapidly enters the central nervous system and has been shown to have much lower toxicological effects compared to MK-801 (3, 29).

Because Dex has not been previously investigated in relation to its effects on the control of breathing or oxygen consumption, the first part of the study consisted of a dose-time-response study in male and female rats. Oxygen consumption was also measured to determine whether Dex had similar effects on both variables, because an increase in ventilation, for example, may have been the consequence of an increase in oxygen consumption. Information from that study was then utilized to determine whether Dex administered before aspartic acid could prevent the aspartic-acid-induced depression of ventilation previously noted in male rats (21, 24). Results of these studies will give us more insight into differential mechanisms associated with the NMDA receptor utilized by male and female rats in modulating the control of breathing.

METHODS

Three-month-old male (n = 12) and female (n = 13) Sprague-Dawley rats obtained from Sasco (Omaha, NE) were utilized in these experiments. The rats were housed three to four animals per cage according to gender for at least 1 wk before the commencement of the experiments. During that time, the animals were handled daily and introduced into the plethysmograph (see below) for a period of 30 min/day. Food and water were available ad libitum. Lighting consisted of 12 h on and 12 h off. All procedures were approved by the University of South Dakota Institutional Animal Use and Care Committee.

Ventilation and oxygen consumption in awake animals were determined with the plethysmographic technique used routinely in my laboratory and described in detail previously (21, 23). Briefly, the plethysmographic chamber consisted of a 19-cm-long 9.5-cm-diam Plexiglas cylinder closed at both ends, with ports to allow air or gases (10% oxygen or 7% carbon dioxide) to enter and exit the chamber. The flow rates of gases exiting the chamber were measured with a Gilmont rotameter. The fractional contents of oxygen or carbon dioxide in the air exiting and entering the chamber were measured with a Beckman OM-14 oxygen analyzer and a Vacumed carbon dioxide analyzer, respectively. Pressure changes associated with ventilation were measured with a low-pressure

THE ROLE OF N-METHYL-D-ASPARTATE (NMDA) RECEPTORS IN MODULATING THE CONTROL OF BREATHING

Modulating the control of breathing has only been studied indirectly. Gender differences associated with NMDA function in the control of breathing have only been studied indirectly. For example, Schlenker and Goldman (22) showed that neonatal treatment of rats with aspartic acid (which acts on NMDA receptors) results in adult male rats that exhibit alveolar hypoventilation and blunted ventilatory responses to hypercapnia. Female rats treated in a similar manner did not exhibit these abnormalities relative to vehicle-treated female rats. In a separate study, these investigators (21) reported that subcutaneous administration of aspartic acid into normal adult rats caused a long-lasting but reversible depression of breathing in male but not in female rats.
ventilatory responses of rats to hypercapnia and hypoxia, the
subcutaneously in volumes of
an injection of Dex followed 30 min later by an injection of saline (Dex/Sal), and
male rats (21) that could be altered by Dex pretreatment.
acid relative to saline caused a depression in breathing in
enced breathing. The difference between the second 15- and
first 30-min reading and the second 15-min reading was
measured. After the second injection, ventilation was
administration of a particular dose of Dex. The rat was then
placed in the chamber, and its ventilation and oxygen con-
sumption were evaluated 15, 30, 45, and 60 min later. Subsequently, the rat was exposed to 7% carbon dioxide in
oxygen (hypercapnia) for 2 min, the chamber was flushed
with air for 15 min, a reading was obtained, and then the rat
was exposed for 2 min to 10% oxygen (hypoxia). Thus each
complete study was ~2 h long. To eliminate effects of circadi-
an variations, each rat was studied at the same time of day.
The results from this study were utilized to design the
following experiment.

To determine the interaction of Dex and aspartic acid
(monosodium salt; Sigma Chemical), the rats received each of
four treatments: 1) an injection of saline followed 30 min later
by another injection of saline (Sal/Sal), 2) an injection of
saline followed 30 min later by an injection of 580 mg/kg of
aspartic acid (Sal/Asp), 3) an injection of 10 mg/kg of Dex
followed 30 min later by an injection of saline (Dex/Sal), and
4) an injection of Dex followed 30 min later by an injection of
aspartic acid (Dex/Asp). All injections were administered
subcutaneously in volumes of ~0.2 ml. After the first injec-
tion, the rat was placed into the chamber, and its ventilation
was measured. After the second injection, ventilation was
measured 15, 30, and 45 min later. The difference between the
first 30-min reading and the second 15-min reading was
evaluated to determine whether Dex relative to saline influ-
enced breathing. The difference between the second 15- and
45-min readings was utilized to determine whether aspar-
tic acid relative to saline caused a depression in breathing in
male rats (21) that could be altered by Dex pretreatment.

Statistical analysis. For the dose-time-response study, venti-
lation, tidal volume, and oxygen consumption were normal-
ized by body weight (1) and expressed in milliliters per
minute per gram for ventilation, in milliliters per gram
multiplied by 100 for tidal volume, and in milliliters per hour
per gram for oxygen consumption. These normalized param-
eters and the others were analyzed with a three-way analysis
of variance (ANOVA) with repeated animals to evaluate the
effects of dose, gender, and time, as well as potential interac-
tions among these factors. If the ANOVA was significant
(P < 0.01), a post hoc least means test (SAS, SAS Institute,
Cary, NC) was used to compare means. A Bonferroni correc-
tion was used for multiple comparisons of the three doses of
Dex compared with the control dose, and significance was set
at P < 0.017. To evaluate the effects of Dex and gender on the
ventilatory responses of rats to hypercapnia and hypoxia, the
ventilation or the preceding air value was subtracted from the
response to the subsequent gas challenge. These changes in
ventilation were analyzed with a two-way ANOVA, and if they
determined to be significant, means were compared with the
tests described above. For the second experiment, a
Wilcoxon’s paired sign test was used to determine whether
Dex affected ventilation relative to saline and whether aspar-
tic acid caused a depression of ventilation relative to saline.

RESULTS

Ventilation was depressed in female rats with Dex at a
dose of 5 mg/kg 15, 30, and 45 min and at a dose of 10
mg/kg 30 min after injection relative to saline (Fig. 1). The
depression of ventilation was the result of a decreased
frequency of breathing at the 5 mg/kg dose (Table 1) and a decrease in tidal volume at both doses
(Table 2). The decrease in ventilation with the 5 mg/kg
dose of Dex was due predominantly to an increase in
inspiratory time at all time points (data not shown).
Expiratory time increased significantly only at 15 min
relative to saline (0.26 ± 0.01 vs. 0.31 ± 0.01 s; P < 0.01). Moreover, frequency tended to decrease with
time (F = 15.2; P < 0.0001). This trend was noted with
all doses of Dex. In contrast, neither tidal volume nor
ventilation exhibited this time-related decrease.

In male rats, Dex had no effect on ventilation while
they were breathing air (Fig. 2). With the exception of the
20 mg/kg dose of Dex at 60 min, neither frequency of
breathing nor tidal volume was affected. Moreover,
unlike female rats, male rats exhibited no time-
dependent decrease in frequency of breathing.

Significant interactions between gender and treat-
ment were noted for ventilation (F = 6.40; P < 0.0003),
frequency of breathing (F = 5.51; P = 0.0011), inspira-
tory time (F = 11.76; P < 0.0001), and expiratory time
Table 1. Breathing frequency of male and female rats treated with dextromethorphan

<table>
<thead>
<tr>
<th>Time, min</th>
<th>0 mg/kg</th>
<th>5 mg/kg</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>131±6</td>
<td>127±6</td>
<td>129±5</td>
<td>117±4</td>
</tr>
<tr>
<td>30</td>
<td>127±7</td>
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<td>125±5</td>
<td>113±4</td>
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<td>45</td>
<td>119±8</td>
<td>114±4</td>
<td>122±4</td>
<td>113±6</td>
</tr>
<tr>
<td>60</td>
<td>123±7</td>
<td>114±4</td>
<td>129±5</td>
<td>104±3*</td>
</tr>
</tbody>
</table>

| Females   |         |         |          |          |
| 15        | 132±6   | 107±4*  | 129±9    | 132±9    |
| 30        | 123±6   | 104±4*  | 115±8    | 119±8    |
| 45        | 108±4   | 91±3*   | 107±6    | 111±5    |
| 60        | 103±4   | 91±3    | 101±6    | 103±8    |

Values are means ± SE in breaths/min. *Significant difference relative to corresponding saline value at that time point, P < 0.001.

(F = 4.30; P = 0.0051). A significant interaction between gender and time was only noted for frequency of breathing (F = 4.63; P = 0.011). No three-way interactions among gender, treatment, and time were observed with any parameter.

Female rats exposed to hypercapnia exhibited a smaller increase in ventilation relative to preceding air values when treated with 5 or 10 mg/kg of Dex relative to saline when treated with 20 mg/kg of Dex. There were no significant interactions between gender and treatment, but each effect alone was significant (gender: F = 6.68; P < 0.01; treatment: F = 12.3, P < 0.0001). Baseline values before the hypercapnic challenge (Fig. 4) showed no effect of Dex in either gender. Relative to saline, Dex treatment did not significantly affect the ventilatory responses of male or female rats to hypoxia (F = 0.48; P = 0.694), but there was a gender effect (F = 5.23; P = 0.0245). Thus the response of male rats overall to hypoxia was greater than that of female rats.

Oxygen consumption was significantly depressed at 20 mg/kg of Dex in female rats at all time points (Fig. 5) and in male rats (Fig. 6) 45 and 60 min after injection. The times and doses at which ventilation and oxygen consumption were depressed did not overlap in female rats or correspond to the changes in ventilation in response to either hypoxia or hypercapnia.

In the second experiment, 10 mg/kg of Dex/Sal relative to Sal/Sal did depress ventilation in female rats (10.56 ml/min for Dex/Sal, P < 0.01; 4.61 ml/min for Sal/Sal, not significant). No such effect of Dex was noted in male rats. The interaction of aspartic acid and Dex is presented in Fig. 7. In male but not in female rats, aspartic acid (Sal/Asp) caused a significant depression in ventilation compared with 15- to 45-min data relative to Sal/Sal. With Dex pretreatment (Dex/Asp), this depression was no longer noted.

DISCUSSION

In the present study, Dex had disparate effects on ventilation in air and in response to hypercapnia in

Table 2. Tidal volume of male and female rats treated with dextromethorphan

<table>
<thead>
<tr>
<th>Time, min</th>
<th>0 mg/kg</th>
<th>5 mg/kg</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>0.19±0.02</td>
<td>0.19±0.01</td>
<td>0.20±0.01</td>
<td>0.20±0.02</td>
</tr>
<tr>
<td>30</td>
<td>0.17±0.01</td>
<td>0.17±0.01</td>
<td>0.19±0.02</td>
<td>0.19±0.01</td>
</tr>
<tr>
<td>45</td>
<td>0.17±0.01</td>
<td>0.17±0.02</td>
<td>0.17±0.01</td>
<td>0.19±0.02</td>
</tr>
<tr>
<td>60</td>
<td>0.19±0.02</td>
<td>0.16±0.01</td>
<td>0.17±0.01</td>
<td>0.18±0.02</td>
</tr>
</tbody>
</table>

| Females   |         |         |          |          |
| 15        | 0.24±0.03 | 0.20±0.02* | 0.21±0.01† | 0.22±0.03 |
| 30        | 0.25±0.02 | 0.19±0.02* | 0.20±0.02† | 0.25±0.03 |
| 45        | 0.24±0.04 | 0.20±0.03 | 0.22±0.03 | 0.24±0.02 |
| 60        | 0.25±0.03 | 0.23±0.03 | 0.23±0.03 | 0.26±0.02 |

Values are means ± SE in ml/g × 100. Significant difference relative to corresponding saline value at that time point: *P < 0.001; †P = 0.022.
females compared with male rats. With 5 and 10 mg/kg of Dex, ventilation was depressed relative to saline in female rats, whereas these doses did not affect ventilation in male rats. The effects of Dex in female rats were due to an increase in inspiratory time that led to a decrease in frequency of breathing and a decrease in tidal volume at the 5 mg/kg dose. Tidal volume also showed a trend to decrease (P = 0.022) at 15 min with the 5 mg/kg dose and at 15 and 30 min with the 10 mg/kg dose. The decrease in ventilation was not due to a concurrent decrease in oxygen consumption. At the highest dose of Dex, oxygen consumption was depressed in both male and female rats. The relative selectivity of Dex on chemoreception was noted in that only hypercapnic but not hypoxic responses were affected. Moreover, a gender-specific response of Dex to hypercapnia was noted.

This is the first study designed to study the effects of Dex, a noncompetitive NMDA-receptor antagonist, on ventilation and oxygen consumption in male and female rats. Aside from a severe overdose, which has been shown to cause profound respiratory depression and even death, other anecdotal accounts of the effects of Dex on ventilation have included tachypnea in male rabbits and human subjects given high doses intravenously (3, 26). Dex is highly lipophilic and quickly enters the brain (29). There it acts on receptors in both the hypothalamus and brain stem regions, including the nucleus of the solitary tract, the nucleus ambiguus, hypoglossal nuclei, and pontine nuclei (6). These re-

**Fig. 4.** Baseline ventilation of male (hatched bars) and female (open bars) rats treated with Dex at various doses before hypercapnic exposure.

**Fig. 5.** Normalized oxygen consumption of female rats given Dex. Open bars, 0 mg/kg of Dex; hatched bars, 5 mg/kg of Dex; solid bars, 10 mg/kg of Dex; stippled bars, 20 mg/kg of Dex. Values are means ± SE of 13 animals per time and dose. *Significantly different from 0 mg/kg dose at that time point after treatment.

**Fig. 6.** Normalized oxygen consumption of male rats given Dex. Open bars, 0 mg/kg of Dex; hatched bars, 5 mg/kg of Dex; crosshatched bars, 10 mg/kg of Dex; solid bars, 20 mg/kg of Dex. Values are means ± SE of 12 animals per time and dose. *Significantly different from 0 mg/kg dose at that time point after treatment.

**Fig. 7.** Decrease in ventilation (15–45 min) in male (hatched bars) and female rats (open bars) given 1 of 4 treatments: saline and then saline (SS), saline and then 580 mg/kg of aspartic acid (SA), Dex (10 mg/kg) and then saline (DS), or Dex before aspartic acid (DA). Values are means ± SE. *Significant decrease in male rats treated with SA compared with those treated with SS. See text for further information.
GENDER EFFECTS OF DEXTROMETHORPHAN ON VENTILATION

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