Blockade of Spontaneous Ovulation by Isocarboxazid (MAO-Inhibitor) in Rats

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Synopsis

Spontaneous ovulation was inhibited in female rats with isocarboxazid (IC), MAO-Inhibitor. Administration of HCG to IC-treated females at 3:00 p.m. on the day of proestrus induced ovulation. Although the brain serotonin level was still high (1.5 times the control) from 9:00 a.m. to 6:00 p.m. on the next day after IC-injection, ovulation occurred on the second day morning. Female rats, with the increased brain serotonin level by IC-injection, copulated on the afternoon of proestrus, and ovulation occurred in the mated animals on the next morning. These results indicate that the stimulus provided by mating may overcome the inhibitory effects of serotonin in IC-treated females.

Materials and Methods

Adult cyclic female rats of the Wistar strain, weighing 200 to 250 g were used in this study. Animals were maintained in controlled condition of light (from 5:00 a.m. to 7:00 p.m.) and temperature (23 ± 1°C). Vaginal smears were taken daily between 9:00 and 10:00 a.m. A history of at least three 4-day cycles in sequence immediately preceding the experimental cycle was required of each rat.

Isocarboxazid (IC: 5-methyl-3-isoxazolecarboxaldehyde 2-benzylhydrazide) was suspended in 0.5% carboxymethyl-cellulose and injected intraperitoneally during proestrus. Controls were administered an equivalent volume (2 ml/kg body weight) of the vehicle. Human Chorionic Gonadotropin (HCG) was injected intraperitoneally to IC-treated females at 3:00 p.m. on the day of proestrus. At each mating a male was placed in each female's cage. Males were introduced into female's cage at 6:00 p.m. on the day of proestrus. Females were exposed to male overnight. The presence of sperm in vaginal smears was accepted as evidence of copulation. At autopsy, the oviducts were removed to examine for ova by the method of Burdick and Whitney (1941).

Method of serotonin determination

Animals were sacrificed by decapitation. A whole brain was removed as rapidly as possible, then homogenized in ice-cold 0.4 N perchloric acid to obtain 50% homogenate by using glass homogenizer with Teflon pestle. Serotonin was measured according to the method of Snyder et al. (1965). After extraction in n-butanol, determination was carried out using Hitachi MPF 2A spectrophotometer at 385 nm for excitation and 490 nm for emission (uncorrected).
Results

Changes in brain serotonin content

In controls, brain serotonin content was found to be 0.510 ± 0.045 μg/g. As shown in Table 1, when rats were injected 30 mg/kg IC at 9:00 a.m. on proestrus (Exp. 1), the brain serotonin level showed a two-fold increase of the control within the first 3 hrs, and reached maximum to 1.041 ± 0.054 μg/g 5 hr later, which were maintained for 12 hr after the administration. A gradual decrease was observed from 1.014 ± 0.023 μg/g (9:00 p.m.) to 0.813 ± 0.080 μg/g (9:00 a.m. on the next day). Afterwards, no remarkable changes were observed for 33 hr (until 6:00 p.m. on the next day) after the administration, while the level showed still 1.5 times higher as compared with that of control.

With 15 mg/kg IC-injection (Exp. 2), the brain serotonin level increased significantly 1.9 times the controls within 5 hr and then gradually decreased to 0.847 ± 0.085 μg/g within 9 hr after the administration of IC.

Effects of isocarboxazid on ovulation

As shown in Table 2, a single injection of 30 mg/kg IC to the animals at 9:00 a.m. on the day of proestrus blocked ovulation (93%) when normal ovulation occurred in controls on the next morning. But, animals treated with IC at 5:00 p.m. on proestrus ovulated normally. A lower dose of 15 mg/kg at 9:00 a.m. blocked ovulation in 9 out of 12 animals.

Fifteen females were treated with IC at 9:00 a.m. on proestrus. Five of them, sacrificed on the second morning (49–51 hr after IC-injection), ovulated. The number of ova showed no significant difference as compared with that of control group (Table 3).

Restoration of ovulation

1) In order to examine the ability of follicles to ovulate in IC-treated animals, HCG was used as an ovulating hormone. After administration of 30 mg/kg IC to ten females at 9:00 a.m. on proestrus, five of them were administered saline and served as controls and other five rats were treated with HCG (30 I.U.) i.p. at 3:00 p.m. on proestrus. All of these saline-treated animals failed to ovulate, while HCG-treated animals ovulated (Table 4).

2) Eleven IC-treated female rats caged with fertile males overnight. Seven out of these 11 females copulated and six out of the mated 7 females ovulated. In contrast, females not mated failed to ovulate (Table 5). These results indicate that coital stimulus could remarkably induce ovulation in females treated with IC.

Table 1. Changes in serotonin content in brain after isocarboxazid administration

<table>
<thead>
<tr>
<th>Time after injection (hr)</th>
<th>Time autopsied</th>
<th>Exp. 1 (30 mg/kg)</th>
<th>P-Value**</th>
<th>Exp. 2 (15 mg/kg)</th>
<th>P-Value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>P*: 9:00 a.m.</td>
<td>0.517 ± 0.386 (6)</td>
<td>n.s.</td>
<td>0.502 ± 0.048 (4)</td>
<td>n.s.</td>
</tr>
<tr>
<td>3</td>
<td>12:00 noon</td>
<td>0.976 ± 0.127 (5)</td>
<td>0.01</td>
<td>0.939 ± 0.046 (4)</td>
<td>0.01</td>
</tr>
<tr>
<td>5</td>
<td>2:00 p.m.</td>
<td>1.041 ± 0.054 (7)</td>
<td>0.01</td>
<td>0.942 ± 0.028 (4)</td>
<td>0.01</td>
</tr>
<tr>
<td>9</td>
<td>6:00 p.m.</td>
<td></td>
<td>0.01</td>
<td>0.847 ± 0.085 (3)</td>
<td>0.01</td>
</tr>
<tr>
<td>12</td>
<td>9:00 p.m.</td>
<td>1.014 ± 0.023 (4)</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>E*: 9:00 a.m.</td>
<td>0.813 ± 0.080 (4)</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>2:00 p.m.</td>
<td>0.783 ± 0.027 (4)</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>6:00 p.m.</td>
<td>0.793 ± 0.014 (4)</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* P: proestrus, E: the next day of proestrus.
** P-Value, difference between experimental and control level (0.510 ± 0.045 μg/g). n.s.: not significant. The numbers of animals used are given in parentheses.
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ISOCARBOXAZID AND OVULATION

Table 2. Effects of isocarboxazid on ovulation

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Time injected</th>
<th>No. rats ovulating</th>
<th>Ova (Mean ± SE)</th>
<th>% Inhibition of ovulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Control*</td>
<td>9:00 a.m.</td>
<td>5/5 (100)</td>
<td>11.2 ± 1.3</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>15</td>
<td>9:00 a.m.</td>
<td>3/12 (25)</td>
<td>11.0 ± 0.8</td>
<td>75</td>
</tr>
<tr>
<td>C</td>
<td>30</td>
<td>9:00 a.m.</td>
<td>2/30 (7)</td>
<td>9.0**</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>11:00 a.m.</td>
<td>3/10 (30)</td>
<td>10.3 ± 4.6</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1:00 p.m.</td>
<td>4/10 (40)</td>
<td>11.0 ± 3.2</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3:00 p.m.</td>
<td>6/10 (60)</td>
<td>11.3 ± 1.7</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5:00 p.m.</td>
<td>10/10 (100)</td>
<td>11.5 ± 1.6</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Animals were administered isocarboxazid during proestrus. Autopsy was done between 10:00 a.m. and 12:00 noon on the next morning after administration of the drug or vehicle.

* Controls were administered an equivalent volume (2 ml/kg) of vehicle.
** Mean value of two animals.

Table 3. Duration of inhibitory effect of isocarboxazid on ovulation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Autopsy*</th>
<th>No. rats ovulating</th>
<th>Ova (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>25-27 hr</td>
<td>3/3 (100)</td>
<td>11.0 ± 1.0</td>
</tr>
<tr>
<td>Isocarboxazid</td>
<td>25-27</td>
<td>0/5 (0)</td>
<td>0</td>
</tr>
<tr>
<td>(30 mg/kg)</td>
<td>49-51</td>
<td>10/10 (100)</td>
<td>11.8 ± 0.5</td>
</tr>
</tbody>
</table>

Isocarboxazid or vehicle was administered to rats at 9:00 a.m. on the day of proestrus.

* Time after administration of the drug or vehicle.
25-27 hr: 10:00 a.m. and 12:00 on the next morning.
49-51 hr: 10:00 a.m. and 12:00 on the second morning.

Table 4. Effects of HCG on ovulation in females pretreated with isocarboxazid

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>No. rats ovulating</th>
<th>Ova (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>0/5 (0)</td>
<td>—</td>
</tr>
<tr>
<td>HCG</td>
<td>5/5 (100)</td>
<td>10.8 ± 0.4</td>
</tr>
</tbody>
</table>

All animals were administered isocarboxazid (30 mg/kg) at 9:00 a.m. on the day of proestrus. Autopsies were done the next morning.

* Saline or HCG (30 I.U.) was administered at 3:00 p.m. on the day of proestrus.

Table 5. Effects of copulation on ovulation in isocarboxazid-treated females

<table>
<thead>
<tr>
<th>No. of females</th>
<th>Ova</th>
<th>Copulating**</th>
<th>Ovulating (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control*</td>
<td>—</td>
<td>2/15*** (13.3)</td>
<td>9.0****</td>
</tr>
<tr>
<td>Mated</td>
<td>7/11 (63.6)</td>
<td>6/7*** (85.7)</td>
<td>10.5 ± 1.8</td>
</tr>
</tbody>
</table>

All animals were administered isocarboxazid (30 mg/kg) at 9:00 a.m. on the day of proestrus. The animals were killed on the next morning.

* Control means only IC-treated females without exposure to male overnight.
** The presence of sperm in vaginal smears was accepted as an evidence.
*** Statistically significant (P < 0.01) when compared with animals not mated.
**** Mean value of two ovulating rats.
Discussion

In the present study, administration of HCG to IC-treated females at 3:00 p.m. on the day of proestrus induced ovulation on the next morning. These results indicate that the inhibitory effects of IC on ovulation are not due to a peripheral but to a central action. Brain serotonin content showed high level during critical period after IC-treatment at 9:00 a.m. on proestrus. Therefore, it is suggested that the increased brain serotonin blocked neural triggers for ovulation.

Everett and Sawyer (1950) suggested that 24-hr periodicity must be a property of the neural component of the LH-release mechanism. They also suggested that failure of the single injection of nembutal on the second day results either from a shortened action of the drug to acquired tolerance or from a more prolonged period of excitability of the LH release system. Although brain serotonin level was high (1.5 times the control) from 9:00 a.m. to 6:00 p.m. on the next day after IC-injection, ovulation occurred on the second morning. These results indicate that the increased brain serotonin failed to inhibit LH-release on the next day. It is assumed that the failure results from an increase in the threshold of trigger mechanism for ovulation to the inhibitory action of serotonin rather than from a more prolonged period of excitability of the LH releasing system.

Female rats with increased brain serotonin level by IC-injection copulated on the afternoon of proestrus, and ovulation occurred on the next morning in the mated females. These results indicate that the stimulus provided by mating may overcome the inhibitory effects of serotonin in IC-treated animals. Labhsetwar (1971b) proposed that the hypothalamus exercises a dual control over ovulation, that is, inhibitory influences being transmitted through serotonin-linked neurons while stimulatory effects are delivered via catecholaminergic fibers to neurons which synthesize releasing factors for ovulating hormones. Although catecholamine levels were not determined quantitatively in this study, it is speculated that the stimulus may activate catecholaminergic fibers and the catecholaminergic system may gain dominance over the serotoninergic system.

Acknowledgements

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References


