Involvement of Nitric Oxide From Nerves on Diarrhea
Induced by Castor Oil in Rats

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ABSTRACT—Nitric oxide (NO) is involved in the mechanism of castor oil-induced diarrhea. This study was performed to elucidate the source of NO. Diarrhea was induced by oral administration of castor oil in rats. Diarrhea was significantly inhibited by the pre-treatment with a relatively selective nerve NO synthase inhibitor, 7-nitroindazole. This effect was attenuated by the treatment with L-arginine. Capsaicin-sensitive afferent nerve degeneration did not affect the diarrhea. N⁶-Nitro-L-arginine methylester significantly inhibited diarrhea even in capsaicin-pretreated rats. These data suggest, at least in part, the involvement of NO from nerves on the diarrhea induced by castor oil in rats.

Keywords: Castor oil, 7-Nitroindazole, Capsaicin-sensitive afferent nerve

Nitric oxide (NO) has been known to play an important role in gastrointestinal function. For example, nitric oxide donating compounds inhibit gastric lesion formation in rats (1). In the intestine, NO is implicated in the cholinergic activation of water secretion. In 1993, Mascolo et al. reported that inhibitors of NO synthase prevented castor oil-induced diarrhea in the rat (2). The present authors also reported the involvement of NO generated by constitutive NO synthase and not by inducible NO synthase on castor oil-induced diarrhea in rats (3). Constitutive NO synthase is divided into two isoforms, nerve NO synthase (4, 5) and endothelium NO synthase (6). It has been known that 7-nitroindazole is not a simple analogue of L-arginine and has a selectivity for neural NO synthase (7). We thus performed the present study to clarify whether nerve NO synthase is the source of NO in castor oil-induced diarrhea by the pharmacological approach.

Capsaicin-sensitive afferent nerve has been known to play an important role in the integrity of gastrointestinal function. Cho and Tang (8) reported that adaptive gastrointestinal protective action by ethanol could act through capsaicin-sensitive afferent nerve with NO and prostaglandin as the possible mediator. Therefore, the effect of capsaicin-sensitive afferent nerve degeneration was also evaluated on diarrhea induced by castor oil.

Crj-CD (Sprague-Dawley) strain male rats weighing about 250 g were purchased from Charles River (Atsugi) and kept for 1 week in a room whose temperature and humidity were kept at 21 ± 2°C and 55 ± 15%, respectively. Before the experiment, rats were fasted for 18 h in meshed cages to prevent coprophagy, but allowed free access to drinking water. Diarrhea was induced by the oral administration of castor oil in a volume of 2 ml/animal. The grade of diarrhea was divided into three categories according to the method of Piercy and Ruwart (9) as follows: Animals were scored for copious (+ +), mild (+) or lack of (0) diarrhea. The total score was calculated by taking the sum of the number of ' + ' rats and twice the number of ' + + ' rats. A score of 0 indicated a complete absence of diarrhea. The observation period was 3 h after castor oil treatment.

Nerve NO synthase inhibitor, 7-nitroindazole (Fumakoshi, Tokyo), suspended in 1% gum arabic solution was administered intraperitoneally at doses of 10 and 30 mg/kg in a volume of 0.1 ml/100 g body weight 30 min before castor oil treatment. In the control rats, the vehicle of 1% gum arabic solution was administered in the same way.

L-Arginine (Wako Pure Chemical, Osaka) at a dose of 600 mg/kg was administered intravenously 15 min after 7-nitroindazole treatment.

Capsaicin-sensitive afferent nerve degeneration was performed according to the method reported by Yonei et al. (10). In brief, capsaicin (Wako Pure Chemical) was dissolved in vehicle consisting of 10% ethanol, 10% Tween 80 (Nacalai Tesque, Kyoto) and 80% saline (vol/vol/vol). Rats received a total dose of 125 mg/kg capsaicin, s.c. over 2 days, with 25 mg/kg in the morning and 50 mg/kg in the
Table 1. Effect of 7-nitroindazole on diarrhea induced by castor oil in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Diarrhea score ++</th>
<th>+</th>
<th>0</th>
<th>Total score (max = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>7-Nitroindazole</td>
<td>10</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>2**</td>
</tr>
</tbody>
</table>

Diarrhea was induced by the oral administration of castor oil in a volume of 2 ml/animal. 7-Nitroindazole suspended in 1% gum arabic solution was administered intraperitoneally at doses of 10 and 30 mg/kg body weight 30 min before castor oil treatment. Five animals were used for each group. Results were analyzed by the cumulative chi-squared test. **: Significant difference from the control group (P<0.01).

Table 2. Effect of l-arginine on the inhibitory effect by 7-nitroindazole on diarrhea induced by castor oil in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Diarrhea score ++</th>
<th>+</th>
<th>0</th>
<th>Total score (max = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>l-Arginine</td>
<td>600</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>7-Nitroindazole + l-Arginine</td>
<td>30</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>4*</td>
</tr>
</tbody>
</table>

Diarrhea was induced by the oral administration of castor oil in a volume of 2 ml/animal. 7-Nitroindazole suspended in 1% gum arabic solution was administered intraperitoneally at doses of 30 mg/kg body weight 30 min before castor oil treatment. l-Arginine at a dose of 600 mg/kg was administered intravenously 15 min after 7-nitroindazole treatment. Five animals were used for each group. Results were analyzed by the cumulative chi-squared test. *: Significant difference from the control group (P<0.05).

Table 3. Effect of capsaicin-sensitive afferent nerve degeneration and N<sup>ω</sup>-nitro-l-arginine methyl ester (L-NAME) on diarrhea induced by castor oil in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Diarrhea score ++</th>
<th>+</th>
<th>0</th>
<th>Total score (max = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle-treated rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>L-NAME</td>
<td>10</td>
<td>6</td>
<td>10</td>
<td>0</td>
<td>10*</td>
</tr>
<tr>
<td>Capsaicin-sensitive afferent nerve degenerated rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>L-NAME</td>
<td>10</td>
<td>4</td>
<td>6</td>
<td>0</td>
<td>14*</td>
</tr>
</tbody>
</table>

Diarrhea was induced by the oral administration of castor oil in a volume of 2 ml/animal. To degenerate capsaicin-sensitive afferent nerve, rats received a total dose of 125 mg/kg capsaicin, s.c. over 2 days. L-NAME dissolved in saline (10 mg/kg) was administered intraperitoneally 15 min before castor oil treatment. Ten animals were used for each group. Results were analyzed by the cumulative chi-squared test. *: Significant difference from control group (P<0.05).

afternoon on the first day and 50 mg/kg once on the second day. The rats were used about 10 days after the treatment with capsaicin. In order to check the effectiveness of the capsaicin treatment, a drop of 0.01% saline solution of capsaicin was instilled into one eye of the rats, and their protective wiping movements were checked. The capsaicin-treated animals that showed any wiping movement were excluded from the study. Control rats received vehicle in the same way. The effect of capsaicin-sensitive afferent nerve degeneration was evaluated on castor oil-induced diarrhea. The effect of N<sup>ω</sup>-nitro-l-arginine methyl ester (L-NAME) was also investigated in vehicle- or capsaicin-treated rats. L-NAME (Sigma, St. Louis, MO, USA) dissolved in saline (10 mg/kg) was administered intraperitoneally 15 min before castor oil treatment. In the control group, saline was administered in the same way.

Statistical analysis was done by the cumulative Chi-squared test and P<0.05 was treated as significant.

The effect of 7-nitroindazole was shown in Table 1. In the control group, diarrhea was induced by castor oil in all rats used and the total score was 9. The treatment with 7-nitroindazole dose-dependently prevented diarrhea, and the total scores at doses of 10 and 30 mg/kg were 7 and 2, respectively. At a dose of 30 mg/kg, a significant difference was observed (P<0.01). l-Arginine itself did not affect the diarrhea (total score was 7), but attenuated the inhibitory effect on diarrhea by 7-nitroindazole and the total score was 7, although a significant difference was not observed as compared with the 7-nitroindazole-treated group (Table 2).

The effect of capsaicin-sensitive afferent nerve degeneration was shown in Table 3. In the control group, diarrhea was induced in all rats used, and the total score was 18. Capsaicin-sensitive afferent nerve degeneration did not affect the degree of diarrhea, and the total score was 20. In vehicle-treated rats, L-NAME significantly inhibited diarrhea (P<0.05), and the total score was 8 (Table 3). Also in the capsaicin-treated group, L-NAME significantly inhibited diarrhea (P<0.05), and the total score was 14 (Table 3).

7-Nitroindazole is a relatively selective inhibitor of nerve NO-synthase (11). In the present study, 7-nitroindazole dose-dependently and significantly prevented the diarrhea induced by castor oil, and this effect was attenuated by the treatment with l-arginine. These findings suggest, at least in part, the involvement of nerve NO synthase in the mechanism of the diarrhea induced by castor oil, although the involvement of endothelial NO synthase was not completely excluded.

There are some reports suggesting that prostaglandin and NO have an interaction between them (12). The present authors reported the involvement of prostaglandin in addition to NO in castor oil-induced diarrhea, since the cyclooxygenase inhibitor indomethacin significantly inhibited the diarrhea (3). On the other hand, capsaicin-sensitive afferent nerve plays an important role to maintain the
integrity of the stomach (13). Cho and Tang reported the correlation between afferent sensory nerve and NO or prostaglandin as possible mediators (8). Brozozowski et al. also reported that the acceleration of the healing and accompanying hyperemia induced by epidermal growth factor at 12 h after water immersion and restrained stress were completely reversed in rats pretreated with L-NAME or in rats with capsaicin-sensitive nerve degeneration (14). In the intestine, Tamai and Gaginella gave more direct evidence showing that NO itself stimulated chloride secretion through a prostaglandin and neural mechanism (15). These reports suggest the correlation between NO and capsaicin-sensitive afferent nerve in the gastrointestinal tract.

On the other hand, 7-nitroindazole is not a simple analogue of L-arginine and exhibits a selectivity for neural NO synthase (7). In the present study, 7-nitroindazole was administered intraperitoneally. There has been no report on the involvement of the central nervous system in the diarrhea induced by castor oil. This point is very interesting. However, to clarify this point, further studies would be needed.

In the present study, capsaicin-sensitive afferent nerve degeneration does not affect diarrhea. On the contrary, L-NAME significantly inhibited diarrhea even in the capsaicin-sensitive afferent nerve degenerated rats. Therefore, it was found that NO released from capsaicin-sensitive afferent nerve presumably does not play a key role in the diarrhea induced by castor oil in rats.

In conclusion, the present study suggested, at least in part, the role of NO released from nerves on the diarrhea induced by castor oil in rats.

REFERENCES