Effects of a New Thyrotropin-Releasing Hormone Analogue Administered in Rats 1 Week After Middle Cerebral Artery Occlusion

Minoru Yamamoto, PhD, Akira Tamura, MD, Takaaki Kirino, MD, Makoto Hirakawa, MD, Masao Shimizu, MS, and Keiji Sano, MD

We observed the effects of a new thyrotropin-releasing hormone analogue, YM-14673 (Nα-[[(S)-4-oxo-2-azetidinyl]carbonyl]-L-histidyl-L-prolinamide dihydrate) on behavioral changes in 26 rats subjected to middle cerebral artery occlusion. The administration of 0.1 mg/kg i.p. YM-14673 was started 1 week after occlusion and was repeated daily for 2 weeks. YM-14673 significantly accelerated the recovery of neurologic deficits and ameliorated the disturbance of passive avoidance learning. Thus, YM-14673 improved behavioral response in a model of chronic focal cerebral ischemia. The availability of a chronic middle cerebral artery occlusion model for the evaluation of drugs is also discussed. (Stroke 1989;20:1089-1091)

There are a number of drugs, such as cerebral metabolism enhancers or cerebral vasodilators, that are currently used during the chronic phase of cerebral vascular diseases. However, the pharmacologies of these drugs have been evaluated using acute focal cerebral ischemia models.1,2 It is of great importance to conduct similar pharmacologic evaluations using a chronic cerebral ischemia model. In previous studies, we observed neurologic deficits and disturbed learning behavior for 4 and 8 weeks after occlusion of the middle cerebral artery (MCA), respectively.3,4 Using this model of chronic cerebral ischemia, neurologic deficits and learning behavior disturbances were ameliorated by the administration of YM-14673, a new thyrotropin-releasing hormone (TRH) analogue (Nα-[[[S]-4-oxo-2-azetidinyl]carbonyl]-L-histidyl-L-prolinamide dihydrate) for 3 weeks immediately after occlusion.5 In addition, YM-14673 showed more potent and longer-lasting facilitatory effects on the central nervous system than did TRH.6,7 The aim of our present study is to describe the effects of YM-14673 on neurologic deficits and disturbed learning behavior in rats with MCA occlusion when administered for 2 weeks, beginning 1 week after occlusion. Since numerous complex physiological changes occur during the acute phase of cerebral ischemia, our present experiment was conducted to exclude a drug effect on unrelated factors operating during acute cerebral ischemia.

Materials and Methods

We conducted the studies using 26 male Wistar rats weighing approximately 300 g. The rats were housed in group cages under 12:12 hour light:dark conditions and were given free access to laboratory food and water. We anesthetized the 26 rats with 2% halothane and permanently occluded the proximal portion of the left MCA in 20 using a microsurgical technique that was modified from our original method developed by Tamura et al8 for the purpose of chronic experiments. The stem of the MCA was electrocauterized just medial to the olfactory tract and was cut to ensure the completeness of the vascular occlusion. Six rats received a sham operation.

In our previous studies, neurologic deficits in rats subjected to MCA occlusion5 were ameliorated by the intraperitoneal administration of YM-14673 at doses of 0.1–0.3 and 0.1–1.0 mg/kg. In the present study, we therefore chose a dose of 0.1 mg/kg i.p. YM-14673. The TRH analogue was prepared in our laboratories and dissolved in saline. We began drug (in 10 MCA-occluded rats) or saline (in 10 MCA-occluded and six sham-operated rats) administration 1 week after occlusion and repeated it daily for 2 weeks. Two persons who did not know to which treatment group a rat had been assigned performed the observations. Neurologic deficits were graded...
FIGURE 1. Chemical structure of YM-14673 and thyrotropin-releasing hormone (TRH).

according to a defined score described by Yamamoto et al.\(^4\) 30 minutes after administration at 1, 2, and 3 weeks after MCA occlusion. The effect of YM-14673 on neurologic deficit was evaluated by the degree of hemiplegia when the right legs of a rat were lifted by a bar and by the degree of abnormal posture when a rat was lifted by its tail. Hemiplegia and posture were graded by the following criteria: 0, no deficit; 1, mild deficit; and 2, severe deficit. Neurologic deficit score was the sum of the hemiplegia and posture grades. Therefore, a score of 0 was the lowest and 4 the highest.

In passive avoidance learning studies, each rat was trained according to the step-through procedure described by Jarvik and Kopp\(^9\); 30 minutes after administration at 1 week after MCA occlusion, each rat was placed in an illuminated safe compartment (40\(\times\)25\(\times\)25 cm) with a hole in the wall through which the rat could enter the dark compartment (20\(\times\)15\(\times\)25 cm), which had a grid on the floor. Once all four paws of the rat were on the grid, a scrambled foot shock (60 V, 50 Hz) was delivered to the grid; the rat could escape the shock only by stepping back into the illuminated safe compartment. In test trials 2 and 3 weeks after occlusion, the rat was placed again in the safe compartment and the response latency (time to enter the dark compartment) was measured during 300 seconds; the response latency of rats that did not enter the dark compartment during the observation period was taken to be 300 seconds.

Neurologic deficit scores and response latencies were averaged for each treatment group. Differences were assessed using the Mann-Whitney U test.

**Results**

We observed a decrease in body weight in the sham-operated and the MCA-occluded groups; the decrease in the MCA-occluded groups was greater than that in the sham-operated group. Among the MCA-occluded rats, the decrease in rats treated with saline was somewhat greater than that in rats treated with YM-14673. Mortality rates during the 3 weeks following occlusion were zero of six, five of 10, and two of 10 in the sham-operated saline-treated, MCA-occluded saline-treated, and MCA-occluded YM-14673-treated groups, respectively. Therefore, the decrease in body weight may be attributed to MCA occlusion, with YM-14673 apparently decreasing mortality. In this behavioral study, we excluded data from rats that died from subsequent analysis.

Maximal neurologic deficits were observed 1 week after occlusion, followed by gradual recovery (Table 1). These changes were similar to those we described previously. YM-14673 significantly accelerated the recovery of neurologic deficits compared with the MCA-occluded saline-treated control group.

Response latency in the MCA-occluded saline-treated rats was shorter than that in the sham-operated group 2 and 3 weeks after occlusion, which is in agreement with the reports described by Tamura et al.\(^3\) and Yamamoto et al.\(^4\) YM-14673 significantly prolonged the response latency compared with the MCA-occluded saline-treated control group (Table 1). Furthermore, in a previous study using normal rats, response latency was not changed by administration of 0.1 mg/kg i.p. YM-14673, indicating that the TRH analogue has no influence on learned behavior in normal rats.\(^10\)

**Discussion**

In our previous study, 0.1 and 0.3 mg/kg i.p. and 1 mg/kg p.o. YM-14673 administered for 3 weeks beginning immediately after MCA occlusion improved neurologic deficits and disturbed learning behavior.\(^4\) Furthermore, our present study also

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Neurologic deficit score</th>
<th>Response latency (sec)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>1 wk</td>
<td>2 wk</td>
</tr>
<tr>
<td>Sham operation</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Occlusion</td>
<td></td>
<td></td>
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<tr>
<td>Saline</td>
<td>5</td>
<td>3.0±0.4</td>
<td>3.4±0.2</td>
</tr>
<tr>
<td>YM-14673</td>
<td>8</td>
<td>3.5±0.3</td>
<td>1.8±0.3*</td>
</tr>
</tbody>
</table>

Data are mean±SEM.

*\(fp<0.05, 0.01\), respectively, different from saline by Mann-Whitney U test.
demonstrates that YM-14673 administered for 2 weeks beginning 1 week after MCA occlusion accelerates recovery of neurologic deficits and ameliorates disturbed learning behavior. It is therefore strongly suggested that YM-14673 possesses beneficial effects on neurologic deficits and disturbed learning behavior. As described in a previous study, the ameliorating effects of YM-14673 may be ascribable to its central facilitatory properties. Furthermore, our present results suggest that YM-14673 may be useful for the treatment of various neurologic symptoms in patients who are chronically recovering from cerebral vascular diseases.

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References

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