WEB 1881 FU Ameliorates Impairment of Working Memory Induced by Scopolamine and Cerebral Ischemia in the Three-Panel Runway Task

Masuo OHNO, Tsuneyuki YAMAMOTO*, Iwao KITAJIMA and Showa UEKI
Department of Pharmacology, Faculty of Pharmaceutical Sciences, Kyushu University 62, Fukuoka 812, Japan

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Abstract—Using a repeated acquisition procedure in a 3-panel runway apparatus, the effect of WEB 1881 FU on impairment of working memory produced either by scopolamine or by cerebral ischemia was investigated in rats and compared with those of aniracetam and Ca hopantenate. Intraperitoneal injection of scopolamine at 0.56 mg/kg significantly increased the number of errors (pushes made on the two incorrect panels of the three panel-gates located at each choice point). WEB 1881 FU at 10-32 mg/kg, p.o., caused a dose-related reduction in the increase of errors expected in the scopolamine-treated rats. Aniracetam at 10-100 mg/kg, p.o., or Ca hopantenate at 100 and 560 mg/kg, p.o., also significantly diminished the increase in errors induced by 0.56 mg/kg of scopolamine. Cerebral ischemia for 5 min significantly increased errors in the 3-panel runway task. WEB 1881 FU at 32 and 56 mg/kg, administered p.o. immediately after blood flow recirculation and again 1 hr before the runway test, conducted 24 hr after ischemia, significantly reduced the increase in errors expected to occur after 5 min of ischemia. Aniracetam at 32 and 100 mg/kg, p.o., similarly diminished the increase in errors in ischemic rats. These findings suggest that WEB 1881 FU has a beneficial effect on memory that has been impaired by scopolamine or by cerebral ischemia.

Recent neurochemical studies on patients with Alzheimer's disease and senile dementia of the Alzheimer type have demonstrated marked reductions in the activity of choline acetyltransferase, the enzyme that synthesizes acetylcholine, in the cerebral cortex and hippocampus (1-3). This suggests an impairment of the cholinergic neurotransmission, which may lead to memory deficits in these pathological states. The administration of scopolamine, a muscarinic acetylcholine receptor antagonist, results in impairment of learning and memory in humans (4-6) and animals (7-11). Neurons in the central nervous system are also vulnerable to ischemia. After cardiac arrest, some patients shows amnesia characterized in part by a reduced ability to learn new and variable information (12, 13). Rats subjected to cerebral ischemia by 4-vessel occlusion (14) exhibit memory deficits (15-17) that are accompanied with central cholinergic dysfunction (18).

WEB 1881 FU (4-aminomethyl-1-benzylpyrrolidin-2-one-fumarate) has an agonistic action on M, -muscarinic acetylcholine receptors in the rat brain (19). It is reported that WEB 1881 FU antagonizes the scopolamine-induced loss of retention of the passive avoidance response in mice (20). Furthermore, WEB 1881 FU exerts a cytoprotective influence, reducing hypoxic lethality in mice, and decreasing the extent of ATP and phosphocreatine depletion in the rabbit brain following cerebral ischemia (20). These findings suggest that WEB 1881 FU has a beneficial effect on memory deficits in animal models of dementia.

In this study, we focused on working memory (21-23). We investigated the effect
of WEB 1881 FU on working memory deficits induced by intraperitoneal scopolamine and cerebral ischemia. These results were compared with two other drugs: another pyrrolidinone derivative, aniracetam; and Ca hopantenate. Both have been shown to improve experimentally induced amnesia (24–26) and loss of cognition in some patients with senile dementia or cerebrovascular disorders (27, 28).

Materials and Methods

Subjects: Eight- to ten-week old male rats of the Wistar strain (SLC) were used in a 3-panel runway task. Initially, their free feeding weights were 230–250 g, but they were maintained at approximately 80% of this level prior to the experiment. The rats were housed in groups of four per cage under a constant temperature (23±2°C) on a 12 hr light-dark cycle (light period: 07:00–19:00) with water freely available.

Apparatus: Working memory was assessed by using a repeated acquisition procedure in a 3-panel runway apparatus (29, 30). In brief, this apparatus (175 x 36 x 25 cm) is composed of a start box, a goal box, and four consecutive intervening choice points. Each choice point consists of a gate with three panels (12 x 25 cm). The rats are prohibited from passing through two of the three panels in the gate by front stoppers and prohibited from returning to the start box or a previous choice point by rear stoppers affixed to each of the panels in all gates. When rats reach the goal box, they get two food pellets (about 50 mg, Muromachi Kikai) as a positive reinforcement.

Acquisition training: Initially, all front stoppers were removed so that a rat could pass through any one of the three panel-gates at each choice point. The rats were made to run the task repeatedly until the time elapsed from leaving the start box to reaching the goal box (latency) fell to consistently below 20 sec. Once rats reached this state they were used in six consecutive trials (one session) per day with the front stopper of only one of the three panel-gates (the correct panel-gate) at each choice point removed. Each trial was performed at 2-min intervals, and water was freely available between trials in the home cage. The locations of the correct panel-gates were held constant within a session, but were changed from one session to the next (working memory). Twelve patterns of correct panel-gate locations were used in this experiment, as described previously (29, 30). The number of times an animal pushed an incorrect panel-gate and the time required for the animal to obtain food pellets were recorded for each rat on every trial of a session. Rats were used for the experiment if they achieved a criterion of less than 12 errors summed across six trials of a session for each of three consecutive sessions.

Scopolamine administration: Scopolamine in a dose of 0.56 mg/kg was administered intraperitoneally to rats that met the criterion. The runway test was performed 20 min after the scopolamine injection. WEB 1881 FU, aniracetam, and Ca hopantenate were administered per os 40 min before scopolamine administration.

Production of ischemia: Cerebral ischemia was induced by the multi-stage method described by Pulsinelli and Brierley (14). The rats were anesthesitized with sodium pentobarbital (35 mg/kg, i.p.), and the vertebral arteries were cauterized bilaterally with a bipolar coagulator (Mizuho Ika, MICRO-ID). Threads were then placed around the common carotid arteries but the carotid blood flow was not interrupted. The next day, rats that behaved normally were used in the final phase of ischemia production. After a runway trial, each rat was restrained in the supine position, and the common carotid arteries were exposed by pulling the threads and were occluded with clips. Rats with vertebral artery cauterization and the carotid artery occlusion lost the righting reflex during the period of ischemia and were assigned to the ischemic group. Control rats had their vertebral arteries cauterized, but their carotid arteries were not occluded. The runway test was performed 24 hr after blood flow recirculation. WEB 1881 FU and aniracetam were administered per os immediately after recirculation and again 1 hr before the runway task.

Drugs: The drugs used in this study were WEB 1881 FU (4-aminomethyl-1-benzyl-pyrrolidin-2-one-fumarate; Nippon Boehringer Ingelheim), aniracetam (Nippon Roche),
Ca hopantenate (Tanabe) and (−)-scopolamine hydrobromide (Sigma). Aniracetam was suspended in 1% carboxymethylcellulose solution, and other drugs were dissolved in distilled water. The doses for drugs were expressed in terms of the salt, except for aniracetam (calculated as the base). Drugs were administered in a volume of 0.1 ml per 100 g body weight.

Data analysis: The number of errors and latency in the first trial were presented separately and summed from the second to sixth trial of a session. The significant difference between groups was determined using a one-way analysis of variance (ANOVA) followed by Dunnett's test when F ratios reached significance (P<0.05).

Results

In the 3-panel runway task, the random performance level is 4 errors per trial and 24 errors per session. With repetition of training, the number of errors made from the second to the sixth trial was markedly decreased, while errors in the first trial remained constant at approximately four (Fig. 1). Almost 20 training sessions were required for rats to achieve a criterion of less than 12 errors summed across six trials of a session. Latency was also reduced with repeated sessions and stabilized on and after the 10th session.

Intraperitoneal injection of 0.56 mg/kg of scopolamine significantly increased errors in the 3-panel runway task [F=49.36, df=1.14], without affecting the number of errors made in the first trial (Fig. 2 and Table 1). Scopolamine prolonged the latency both in the first trial [F=6.47, df=1.14] and from the second to sixth trial [F=19.46, df=1.14]. WEB 1881 FU at doses of 10–32 mg/kg caused a dose-related reduction in the increase of errors expected in the scopolamine-treated rats [F=4.18, df=3,28]. WEB 1881 FU had no effect on the increase in latency. As shown in Table 1, the increase of errors induced by 0.56 mg/kg of scopolamine was also significantly reduced by aniracetam at 10–100 mg/kg [F=3.10, df=3,28] as well as Ca hopantenate at 100 and 560 mg/kg [F=8.90, df=2,21]. However, the prolonged latency in the scopolamine-injected rats was unaffected by these doses of aniracetam or Ca hopantenate.

The five-minute period of cerebral ischemia led to a significant increase in the number of
errors in the 3-panel runway task \(F=87.47, \text{df}=1,10\), without affecting errors made in the first trial (Fig. 3 and Table 2). Ischemia prolonged the latency in the first trial \(F=8.81, \text{df}=1,10\) and from the second to sixth trial \(F=74.58, \text{df}=1,10\). The administration of 32 and 56 mg/kg of WEB 1881 FU immediately after blood flow recirculation and then again 1 hr before the test session held the next day significantly reduced the increase in errors that would normally be expected after 5 min of ischemia \(F=16.42, \text{df}=2,15\). Treatment with these doses of WEB 1881 FU failed to decrease the expected increase in latency. As shown in Table 2, aniracetam at doses of 32 and 100 mg/kg also caused a significant reduction in the increase of errors induced by 5 min of ischemia \(F=7.81, \text{df}=2,15\). Aniracetam at 100 mg/kg decreased the number of errors in the first trial \(F=5.44, \text{df}=2,15\). A significant reduction of the prolonged latency was seen when rats were treated with 32 mg/kg of aniracetam \(F=9.30, \text{df}=2,15\).

**Discussion**

There is much evidence that central cholinergic function plays a crucial role in learning and memory. For instance, a muscarinic receptor agonist such as oxotremorine or arecoline, and the acetylcholinesterase inhibitor physostigmine facilitate learning and memory in humans (6, 31) and animals (32). Conversely, scopolamine, a muscarinic antagonist, has been shown to produce amnesic effects in humans (4–6) and in mice and rats subjected to a passive avoidance test (7) or a radial maze task (8, 11). Similarly, scopolamine causes a disruption of working memory, as indicated by an increase in errors in the 3-panel runway task (29, 30).

Our study clearly showed that WEB 1881 FU reversed impairment of working memory induced by scopolamine. This result is consistent with the fact that WEB 1881 FU antagonizes scopolamine-induced amnesia in the passive avoidance task in mice (20). Muscarinic agonists such as oxotremorine and arecoline have been found to block the amnesic effects of scopolamine (33). Kitamura et al. (19) demonstrated in their binding experiment with \(^{3}H\)pirenzepine that WEB 1881 FU directly binds to M1- muscarinic receptors in the rat brain. They
Table 1. Effects of WEB 1881 FU, aniracetam, and Ca hopanenate on 0.56 mg/kg of scopolamine-induced increases in errors and latency in the 3-panel runway task

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Drug</th>
<th>mg/kg (p.o.)</th>
<th>N</th>
<th>Number of errors</th>
<th>Latency (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Trial 1</td>
</tr>
<tr>
<td>Control</td>
<td>—</td>
<td>—</td>
<td>8</td>
<td>3.9±0.4</td>
<td>4.1±0.9</td>
</tr>
<tr>
<td>Scopolamine</td>
<td>—</td>
<td>—</td>
<td>8</td>
<td>4.4±0.2</td>
<td>18.3±1.8**</td>
</tr>
<tr>
<td>(0.56 mg/kg)</td>
<td>WEB 1881 FU</td>
<td>10</td>
<td>8</td>
<td>3.8±0.5</td>
<td>16.0±1.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18</td>
<td>8</td>
<td>4.5±0.3</td>
<td>13.4±1.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>32</td>
<td>8</td>
<td>3.9±0.4</td>
<td>8.8±2.6**</td>
</tr>
<tr>
<td>Scopolamine</td>
<td>Aniracetam</td>
<td>10</td>
<td>8</td>
<td>4.4±0.7</td>
<td>16.6±1.3</td>
</tr>
<tr>
<td>(0.56 mg/kg)</td>
<td></td>
<td>32</td>
<td>8</td>
<td>4.1±0.4</td>
<td>11.1±1.8*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>8</td>
<td>4.5±0.6</td>
<td>12.0±2.7*</td>
</tr>
<tr>
<td>Scopolamine</td>
<td>Ca hopanenate</td>
<td>100</td>
<td>8</td>
<td>4.4±0.5</td>
<td>12.6±2.0*</td>
</tr>
<tr>
<td>(0.56 mg/kg)</td>
<td></td>
<td>560</td>
<td>8</td>
<td>3.8±0.3</td>
<td>8.4±1.0**</td>
</tr>
</tbody>
</table>

WEB 1881 FU, aniracetam, and Ca hopanenate were administered 40 min before injection of scopolamine, which was performed 20 min prior to the runway test. Each value represents the mean±S.E. The significance of differences from control rats (⁎P<0.05, ⁎⁎P<0.01) and from scopolamine-treated rats (⁎P<0.05, ⁎⁎P<0.01) was determined using a one-way ANOVA followed by Dunnett’s test.

Table 2. Effects of WEB 1881 FU and aniracetam on the increase in errors and latency induced by a 5-min period of cerebral ischemia in the 3-panel runway task

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Drug</th>
<th>mg/kg (p.o.)</th>
<th>N</th>
<th>Number of errors</th>
<th>Latency (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Trial 1</td>
</tr>
<tr>
<td>Control</td>
<td>—</td>
<td>—</td>
<td>6</td>
<td>3.7±0.4</td>
<td>2.3±0.6</td>
</tr>
<tr>
<td>Ischemia</td>
<td>—</td>
<td>—</td>
<td>6</td>
<td>4.7±0.3</td>
<td>16.5±1.4**</td>
</tr>
<tr>
<td>(5 min)</td>
<td>WEB 1881 FU</td>
<td>32</td>
<td>6</td>
<td>4.3±0.3</td>
<td>8.5±1.1**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>56</td>
<td>6</td>
<td>3.7±0.4</td>
<td>7.0±1.3**</td>
</tr>
<tr>
<td>Ischemia</td>
<td>Aniracetam</td>
<td>32</td>
<td>6</td>
<td>4.2±0.3</td>
<td>8.2±1.6**</td>
</tr>
<tr>
<td>(5 min)</td>
<td></td>
<td>100</td>
<td>6</td>
<td>3.3±0.2**</td>
<td>11.7±1.5*</td>
</tr>
</tbody>
</table>

WEB 1881 FU and aniracetam were administered immediately after blood flow recirculation and again the next day, 1 hr before the runway test. Each value represents the mean±S.E. The significance of differences from control rats (⁎⁎P<0.01) and from ischemic rats (⁎P<0.05, ⁎⁎P<0.01) was determined using a one-way ANOVA followed by Dunnett’s test.
suggest that WEB 1881 FU acts as an agonist on M₁-muscarinic receptors because the WEB 1881 FU-competition curve for [³H]-pirenzepine binding to hippocampal membranes was rightward-shifted by GTPßS, as observed in the case of oxotremorine, and because long-term administration of WEB 1881 FU induced the down-regulation of M₁-muscarinic receptors in the hippocampus and striatum. Recently, it has been reported that intracerebroventricular injection of pirenzepine, a selective M₁-muscarinic antagonist, impairs passive avoidance learning in mice (34) and spatial learning in a water maze in rats (35, 36), which suggests an important role for central M₁-muscarinic receptors in learning and memory. Therefore, it is conceivable that the ameliorating effect of WEB 1881 FU on scopolamine-induced amnesia is attributable to its agonistic action on central M₁-muscarinic receptors. Another pyrrolidinone derivative, aniracetam, and Ca hopantenate also antagonized scopolamine-induced impairment of working memory in the runway task. Aniracetam has been found to reverse amnesia in passive avoidance tasks and the decrease in acetylcholine level in the hippocampus produced by scopolamine in rats (24, 25). Ca hopantenate, which consists of γ-amino butyric acid (GABA) and pantoyl moieties, binds GABA receptors, causing enhancement of acetylcholine synthesis and release in cholinergic terminals in the cerebral cortex and hippocampus (37, 38). This suggests that some stimulant action of aniracetam or Ca hopantenate on the central cholinergic system may play a role in the improvement of memory deficits produced by scopolamine.

The central nervous system is one of the systems most vulnerable to ischemia. Rats subjected to cerebral ischemia by 4-vessel occlusion (14) exhibit memory deficits, as assessed in passive avoidance tasks (17) and 8-arm radial mazes (15, 18). In our experiment, impairment of working memory in the 3-panel runway task was successfully produced in rats by a 5-min period of cerebral ischemia. WEB 1881 FU as well as aniracetam administered immediately after recirculation and again before a test session conducted 24 hr after ischemia, ameliorated the ischemia-induced amnesia. Aniracetam has also been shown to protect against the disruption of memory in an active avoidance task induced in mice by exposure to CO₂ (24). However, the mechanisms underlying the activity of aniracetam remain unresolved. In our previous study (30, 39), cholinesterase inhibitors such as physostigmine, tetrahydroaminoacridine, and amiridin reversed amnesia in the runway task in ischemic models. Yamazaki et al. (17) have reported that physostigmine reverses the ischemia-induced amnesia in the passive avoidance task when it was administered only once before the retention test conducted 24 hr after the ischemia. Pretreatment with physostigmine also protects against the lethal effect of hypoxia in mice (40, 41). Furthermore, the cholinergic dysfunction, i.e., a significant decrease in acetylcholine and a marked increase in choline, has been noted in the brain of rats with ischemia (18). These findings suggest that the agonistic action of WEB 1881 FU on central M₁-muscarinic receptors may be related to the amelioration of memory deficits induced by cerebral ischemia, although it is not clear which of the effects of two administrations plays a more important role. Cerebral ischemia also causes a marked reduction of brain ATP and phosphocreatine levels in rats (42) and gerbils (43). A recent biochemical study (20) has shown that pretreatment with WEB 1881 FU for 7 days diminishes the breakdown of ATP and phosphocreatine concentrations in the rabbit brain following cerebral ischemia. Therefore, it is conceivable that the memory deficit reversal produced by WEB 1881 FU may result from protection of the brain from the energy failure induced by cerebral ischemia. This is predominantly attributable to the effect of WEB 1881 FU administered immediately after recirculation, because the high-energy phosphates are rapidly restored to normal levels during the early postischemic period (42, 43).

We conclude that WEB 1881 FU reverses scopolamine-induced amnesia through its agonistic action on central M₁-muscarinic acetylcholine receptors. Both the cholinergic activating action of WEB 1881 FU and its beneficial effect on energy metabolism may play a role in the amelioration of memory deficits in the ischemic model.
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