Focal Cerebral Ischemia-Induced Escape Deficit in Rats Is Ameliorated by a Reversible Inhibitor of Monoamine Oxidase-A: Implications for a Novel Animal Model of Post-Stroke Depression

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The present investigation was conducted to examine whether a reversible inhibitor of monoamine oxidase (MAO)-A, T-794, affects the shuttle-box escape deficit induced by transient middle cerebral artery (MCA) occlusion (MCAO). MCA-occluded and sham-operated rats (surgery on day 0) were subjected to daily shuttle-box session from day 7 to 9 (training series) and from day 13 to 15 (test series) and received twice daily administration of T-794 (10 mg/kg p.o., b.i.d.) or vehicle from the evening of day 9. In the final shuttle-box session of test series (day 15), while MCA-occluded–vehicle-treated rats showed significantly more escape failures than sham-operated–vehicle-treated rats, the failures made by MCA-occluded rats were significantly decreased by T-794 to the level of the sham-operated group. Additionally, biochemical examination was conducted after behavioral evaluation to examine possible involvement of the brain monoamine system in the observed behavioral syndromes. In occluded hemisphere of MCA-occluded rats, catecholamine levels were decreased and ratios of deaminated metabolite to corresponding monoamine were increased compared with the respective values of the sham-operated group, and these changes were reversed by T-794. Results are discussed in terms of possible relevance of the MCAO-induced escape deficit to post-stroke depression.

Key words: middle cerebral artery occlusion; monoamine oxidase-A inhibitor; post-stroke depression; T-794; behavior

Although various psychiatric syndromes including depression are common after stroke, the etiology of development is not fully understood. Thus, there is great demand for animal studies which can bring more understanding of these matters. In a pilot study, we discovered that rats which received transient middle cerebral artery (MCA) occlusion (MCAO) show poor escape performance in the shuttle-box compared with non-occluded control, even after they recovered from acute neurological symptoms.

It is well-known that inescapable stress induces shuttle-box escape deficit, which is reversed by repeated administration of antidepressant agents. We also reported that drugs including imipramine and a novel reversible inhibitor of monoamine oxidase-A (MAO-A), T-794, reversed such escape deficit. The escape deficit following MCAO, which we recently discovered in the pilot study, was observed in the same apparatus and under the same conditions of shuttle-box as the previous report, in which the drugs reversed the inescapable stress-induced deficit. This led us to examine the hypothesis that T-794 also reverses the escape deficit induced by MCAO.

In the pilot study, the MCAO-induced escape deficit became more obvious when subjects experienced the shuttle-box sessions repeatedly, rather than after a single session. Thus, we set up a protocol to repeat the sessions for 3 consecutive days each, both before and after drug administration: before the administration for assignment of subjects equally into groups (training series) and after the administration for evaluation of drug effect (test series). In addition, brain monoamine concentration was measured at the end of the experiment to examine the possible involvement of the brain monoamine system in the observed behavioral syndrome.

T-794 is a novel inhibitor of MAO-A whose inhibitory action was shown in vivo as well as in vitro and ex vivo. It was effective in various animal models of depression and its effect was also demonstrated in the behavioral despair test using rats subjected to global cerebral ischemia.

MATERIALS AND METHODS

Animals Male CD rats (8 weeks, Charles River Japan, Yokohama, Japan) were used in the studies. All animals were purchased 1–2 weeks before use in the experiment, and housed at constant room temperature (23±1 °C) and relative humidity (55–70%) under 12 h light–dark cycle (lights on at 7:00 A.M.). Food was deprived for one night before the day of surgery; otherwise food and water were available ad libitum. Rats were handled twice before the surgery. The experimental protocol used in the present study followed guiding principles for the care and use of laboratory animals (approved by the Japanese Pharmacological Society) and was approved by the Ethical Committee of Tanabe Seiyaku Co., Ltd.

Drugs T-794 was synthesized at Tanabe Seiyaku. Other drugs were purchased from commercial sources: 5-hydroxytryptamine (5-HT) creatininesulfate, dopamine (DA) hydrochloride, 3-methoxy-4-hydroxyphenylglycol (MHPG) hemipiperazine salt, MHPG-SO4 potassium salt, 5-hydroxyindoleacetic acid (5-HIAA), 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) from Sigma (St. Louis, MO, U.S.A.), l-norepinephrine (NE) from Katayama Chemicals (Osaka, Japan), 1-octanesulfonate sodium from Nacalai Tesque (Kyoto, Japan). T-794 was suspended in 0.5% carboxymethylcellulose Na (CMC) and administered in a volume of 5 ml/kg.

MCAO Transient occlusion of the left MCA was induced by the method of Nagasawa et al. Briefly, after a median incision of the neck skin, the left carotid artery was exposed. A piece of nylon thread, distal 5 mm of which was
coated with silicone resin, was inserted from the bifurcation of the internal carotid artery to occlude the origin of the left MCA, immediately after ligation of the ipsilateral common and external carotid arteries. Then, the internal carotid artery was ligated just distal to the point of insertion. After 2 h of occlusion, recirculation was performed by pulling the thread out of the internal carotid artery. During the surgery of both occlusion and recirculation, rats were anesthetized with 1—1.5% halothane and their rectal temperature was maintained at 37±1°C by a heating pad. For sham operation, identical surgery was performed except for the insertion of thread. Day of the surgery was defined as day 0.

**Neurological Examination** Neurological symptoms were evaluated according to the method of Barone et al. at 24 h (day 1) and 7 d (day 7) after the MCAO. Each animal was classified as one of following neurological grades: 0 (no observable deficit), 1 (any amount of consistent contralateral forelimb flexion), 2 (reduced resistance to lateral push toward the contralateral side), 3 (circling behavior toward the contralateral side) (ipsilateral and contralateral were referred based on the occluded side). A hindlimb placement test was also performed. In this test, the rat was held facing away from the edge of a table, and the contralateral hindlimb is pulled over the edge of the table and extended downward. A normal response seen in intact animals or in the ipsilateral hindlimbs is an immediate placement of the hindlimb back onto the table and an abnormal response is no limb placement.

**Behavioral Evaluation in Shuttle-Box** On day 7, 8, 9 (training series), and on day 13, 14, 15 (test series), the rats were evaluated for their escape behavior in automated two-way shuttle-boxes (450×200×191 cm, Neuroscience, Inc., Tokyo, Japan); on day 7, the session was held just after the neurological examination. The schedule for the shuttle-box sessions were described previously. In brief, rats were individually placed in a shuttle-box and allowed to habituate to the environment for 5 min, and then subjected to 30 shuttle trials with a fixed intertrial interval of 30 s. In each trial, a tone signal (80—90 dB) was firstly presented for a maximum of 3 s duration; rats were allowed to avoid the electric shock by moving to the other side of the box (avoidance response). If no avoidance response occurred within 3 s, a scrambled electric shock (0.8 mA) was applied thorough the grid floor for a maximum of 3 s duration; rats were allowed to escape from the shock by moving to the other side of the box (escape response). If no escape response occurred within 3 s, shock and tone terminated automatically (escape failure). The number of escape failures per 30 trials was considered as the index of escape performance. We assigned MCA-occluded rats into 2 groups (T-794-treated, n=8; vehicle-treated, n=8) according to the performance in the last session of the training series (day 9) and set the endpoint for the behavioral evaluation on the performance in the last session of the test series (day 15).

**Measurement of Spontaneous Motor Activity (SMA)** On day 14, a day before the endpoint evaluation of escape behavior, SMA of rats was measured. From 15 min before the shuttle-box session starts, rats were individually placed in plexiglass chambers (45×45×35 cm), where they were left free to move for 10 min, and their SMA was measured using a movement analyzing system (SCANET®, Toyo Sangyo Co., Ltd., Toyama, Japan). This system traces and counts the animal's movement by scanning 144 pairs of photocells on the side walls every 0.1 s. Then, rats were returned to the home-cage.

**Measurement of Monoamines and Their Metabolites** On the day after the last shuttle-box session (day 16), rats were decapitated 1 h after the last drug administration, and brain was removed. Whole brain except the cerebellum and olfactory bulb was divided into right and left hemisphere on ice, and the tissues were frozen by dry ice and maintained at −80°C until measurement. The samples were homogenized by a sonicator (Nissei, Tokyo, Japan) in 0.2 n HClO (2.5 ml) containing 0.05 M EDTA-2Na (50 μl) and centrifuged (25000×g, 20 min, 4°C). An aliquot of the supernatant was injected to a high-performance liquid chromatograph with electrochemical detection (EP-10, Eicom, Kyoto, Japan) to measure the concentrations of 5-HT, 5-HIAA, NE, DA, DOPAC and HVA. For the measurement of MHPG, the MHPG-SO4, was hydrolyzed by the method of Fukushima et al. A reversed phase separation column (Eicompac MA-5-ODS, Eicom, Kyoto, Japan) was used and the mobile phase consisted of 0.0415 m sodium acetate—0.0415 m citric acid buffer (pH 3.6) containing 17% methanol, 220 mg/l 1-octanesulfonate-Na and 40 mg/l EDTA-2Na. The flow rate of the mobile phase was kept at 0.8 ml/min for the measurement of 5-HT, 5-HIAA, NE, DA, DOPAC and HVA or 0.7 ml/min for MHPG.

**Drug Treatment** From the evening of day 9 to the morning of day 16, rats were orally administered with T-794 (10 mg/kg per administration) or vehicle (CMC), twice daily in the morning (8:30—9:30) and the evening (17:30—18:30), except for the days of shuttle-box sessions or decapitation, when the administration in the morning was held 1 h before the treatment.

**Statistics** Results are shown as mean with standard error of the mean. Comparison of groups were performed by one-way ANOVA followed by Fisher’s PLSD test. Differences at a p value of less than 0.05 were considered to be statistically significant.

**RESULTS**

**Neurological Symptoms** On the day after surgery, i.e. day 1, the mean neurological grade of MCA-occluded animals (n=16) was 0.50±0.18 and, in the hindlimb placement test, 43.8% showed abnormal response. In contrast, on day 7, neurological grades of all the occluded rats were 0 and abnormal response in the hindlimb test was observed in 12.5% of rats, indicating that the neurological symptoms of MCA-occluded rats had essentially disappeared by the beginning of the training series of shuttle-box sessions. In the sham-operated group, all subjects showed 0 in the neurological grade and none of them showed abnormal response in the hindlimb placement test, both on day 1 and 7.

**Escape Behavior in the Shuttle-Box** In the final shuttle-box session of the training series (day 9), the number of escape failures per 30 trials of MCA-occluded and sham-operated rats were 7.1±2.5 and 1.3±1.0, respectively; although MCA-occluded animals had more failures than sham-operated animals, this difference was not statistically significant. Just after the shuttle-box session on day 9, MCA-occluded
rats were assigned into vehicle-treated (MCAO–CMC) and T-794-treated (MCAO–T-794) groups, so that the mean number of failures of the 2 groups were roughly equal (Fig. 1A). In the final session of the test series (day 15), the MCAO–CMC group had significantly more failures than the sham-operated–vehicle-treated (sham-CMC) group. Moreover, the MCAO–T-794 group had significantly less failures than the MCAO–CMC group (Fig. 1B). It is also interesting to note that the MCAO–CMC group tended to have more failures in the test session (day 15) than in the training session (day 9).

SMA Mean SMA (±S.E.M.) of sham–CMC, MCAO–CMC and MCAO–T-794 groups, measured 15 min before the shuttle-box session, i.e. 45 min after the drug administration, on day 14, were 819.8±311.7, 1312.1±590.5 and 1280.6±253.0 counts per 10 min, respectively. Although MCA-occluded groups tended to show higher SMA than the sham–CMC group, there was no significant difference between groups.

Monoamines and Their Metabolites The monoamine concentrations and the ratios of deaminated metabolite concentration to that of the corresponding monoamine (turnover ratios) in the brain are shown in Figs. 2 and 3, respectively. MCAO decreased NE and DA concentrations and increased the turnover ratios of all 3 monoamines in the occluded hemisphere. In contrast, in that hemisphere, the MCAO–T-794 group showed higher concentrations and lower turnover ratios of all 3 monoamines than the MCAO–CMC group. In the non-occluded hemisphere, MCAO affected neither the

![Fig. 1. Effect of T-794 on MCAO-Induced Escape Deficit in Rats (Occlusion on Day 0)](image)

Data represent means±S.E.M. of escape failures per 30 shuttle-box trials on day 9 (A, training series) and on day 15 (B, test series). MCA was occluded on day 0 and T-794 (10 mg/kg) or CMC was orally administered twice daily from the evening of day 9. *p<0.05, comparison by one-way ANOVA, followed by post-hoc comparison. n=6 in sham–CMC group, n=8 in others.

![Fig. 2. Effect of MCAO and T-794 (10 mg/kg, b.i.d.) on the Levels of Monoamines in Rat Brain](image)

T-794 or CMC was orally administered twice daily from the evening of day 9 and animals were sacrificed 1 h after the last administration in the morning on day 16. *p<0.05, ##p<0.01 vs. sham-CMC group, †p<0.05, ††p<0.01 vs MCAO-CMC group, comparison by one-way ANOVA, followed by post-hoc comparison. n=6 in sham–CMC group, n=8 in others.
monoamine concentrations nor the turnover ratios; T-794 increased 5-HT concentration and decreased the turnover ratios of all 3 monoamines.

DISCUSSION

The present experiment was conducted to examine the hypothesis that T-794, a reversible inhibitor of MAO-A, can affect MCAO-induced shuttle-box escape deficit, the syndrome which we recently discovered in our pilot study. The results clearly produced an answer to this hypothesis; twice daily administration of T-794 at a dose of 10 mg/kg p.o. reverses the development of escape deficit induced by MCAO. Although these results provide important information to understand the nature of observed escape deficit, careful consideration should be necessary on factors involved in the development and the reversal of the escape deficit.

Firstly, it is necessary to consider the possibility that the escape deficit was induced simply by the change in SMA, since it is well known that low SMA results in poor escape performance in the shuttle-box. However, this is not likely because MCAO-occluded rats tended to show higher SMA values than sham-operated rats, although statistically non-significant, and T-794 did not affect the SMA of animals, at day 14, a day before the end-point measurement of escape performance. No interaction on SMA by T-794 was consistent with previous results that it did not affect SMA up to 100 mg/kg p.o. in mice (unpublished data) and with twice daily administration of 30 mg/kg p.o. in rats subjected to global ischemia.

Secondly, the observed escape deficit might be derived from the change in pain threshold, since the magnitude of aversive stimulus may well influence the motivation to escape. This factor cannot be completely eliminated from the present results alone, but the following lines of evidence argue against this explanation: MCAO-occluded rats showed essentially no sensory deficit in the neurological examination on day 7, when the training series were about to start. Moreover, reversal of the escape deficit by T-794 does not seem to be due to a change in the pain threshold either, because our previous experiment demonstrated that T-794 has no effect on the pain threshold up to 100 mg/kg p.o. in mice, assessed in the acetic acid-induced writhing test (unpublished data).

Another possible explanation is that cognitive deficit is involved in the development of escape deficit. It is widely accepted that experimental ischemia negatively affects cognitive function and thus this may be a reasonable explanation. However, we do not have any data on whether T-794 can improve cognitive ability.

It is also possible that the MCAO-induced escape deficit is derived from not only cognitive but also motivational or emotional deficit, like so-called 'learned helpless' behavior; learned helplessness is a well recognized animal model of
depression which is caused by inescapable stress. The following lines of evidence are supportive to this explanation: the MCAO-induced escape deficit was observed using the same shuttle-box conditions as we detected the inescapable stress-induced escape deficit which is reversed by drugs including imipramine; T-794, which is effective in various animal models of depression, reversed the MCAO-induced escape deficit.

Depression is a common and serious complication after cerebral stroke and is referred to as post-stroke depression. Thus, the behavioral change which reflects such a clinical syndrome may well occur in rats subjected to experimental ischemia. If the shuttle-box escape deficit observed in the present study is due to cognitive, motivational and/or emotional deficit caused by MCAO, it is possible that this deficit has relevance to post-stroke depression. However, we cannot draw conclusions from present results alone and more experiments are necessary for further discussion on this matter.

At the end of behavioral examination, the concentration of brain monoamines and their metabolites was measured in order to elucidate whether the brain monoamine system has relevance to the escape deficit induced by MCAO. Our present study demonstrated that MCAO increases the concentration of brain monoamines except 5-HT and decreases the turnover ratios in the occluded hemisphere. On the other hand, T-794 reversed these changes; it increased brain monoamine concentration and decreased turnover ratio. These results seem to support the notion that the brain monoamine system is involved in the development of escape deficit. However, we could not find a correlation between the biochemical indices (monoamine levels or turnover ratios) and the behavioral performance (the number of failures) of the subjects. We have to wait for additional experiments to clarify the neurobiological basis which underlie the observed behavioral syndrome.

Although further investigations are necessary to elucidate whether the MCAO-induced shuttle-box escape deficit is related to some clinical syndrome following the cerebral stroke or what mechanism underlies the development of the deficit, we believe that the present study provides new and interesting evidence on the behavior of animals which were subjected to focal cerebral ischemia.

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REFERENCES