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Chronic administration of citalopram inhibited El mouse convulsions and decreased monoamine oxidase-A activity.

Hideaki Kabuto* Isao Yokoi† Atsushi Endo‡
Mineo Takei** Tadashi Kurimoto†† Akitane Mori‡‡

*Okayama University,
†Okayama University,
‡Okayama Univeristy,
**Okayama University,
††Zeria Pharmaceutical company limited,
‡‡Okayama University,

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Hideaki Kabuto, Isao Yokoi, Atsushi Endo, Mineo Takei, Tadashi Kurimoto, and Akitane Mori

Abstract

Serotonin (5-HT) is thought to play an important role in the seizures of El mice because the seizure threshold of El mice correlates with the 5-HT concentration in the central nervous system. In this study, the anticonvulsant effect of a 5-HT reuptake blocker, citalopram, was evaluated behaviorally and biochemically. El mouse convulsions were inhibited by chronic administration of citalopram (80 mg/kg/day, p.o. for 2 weeks), but were not inhibited by acute administration of citalopram (80 mg/kg, i.p., 2 h after single injection). Both chronic and acute administration of citalopram decreased the concentration of 5-hydroxyindolacetic acid in the brain, whereas the concentration of 5-HT was not changed by treatment with citalopram. Tryptophan hydroxylase activity was not different between the citalopram and control groups, although the monoamine oxydase-A activity was lowered by chronic administration of citalopram. These findings suggest that both acute and chronic administration of citalopram depresses the 5-HT turnover rate, however chronic administration is necessary to inhibit El mouse convulsions.

KEYWORDS: citalopram, serotonin, MAO-A, Trp-OHase, EL mouse

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Chronic Administration of Citalopram Inhibited El Mouse Convulsions and Decreased Monoamine Oxidase-A Activity

HIDEAKI KABUTO*, ISAO YOKOI, ATSUSHI ENDO, MINEO TAKEI[a], TADASHI KURIMOTO[a] AND AKITANE MORI

Department of Neuroscience, Institute of Molecular and Cellular Medicine, Okayama University Medical School, Okayama 700, Japan and [a]Department of Pharmacology, Zeria Pharmaceutical Co., Ltd. 2512-1, Oshikiri, Kohnan-machi, Ohsato-gun, Saitama 360-01, Japan

Serotonin (5-HT) is thought to play an important role in the seizures of El mice because the seizure threshold of El mice correlates with the 5-HT concentration in the central nervous system. In this study, the anticonvulsant effect of a 5-HT reuptake blocker, citalopram, was evaluated behaviorally and biochemically. El mouse convulsions were inhibited by chronic administration of citalopram (80 mg/kg/day, p.o. for 2 weeks), but were not inhibited by acute administration of citalopram (80 mg/kg, i.p., 2 h after single injection). Both chronic and acute administration of citalopram decreased the concentration of 5-hydroxyindolacetic acid in the brain, whereas the concentration of 5-HT was not changed by treatment with citalopram. Tryptophan hydroxylase activity was not different between the citalopram and control groups, although the monoamine oxydase-A activity was lowered by chronic administration of citalopram. These findings suggest that both acute and chronic administration of citalopram depresses the 5-HT turnover rate, however chronic administration is necessary to inhibit El mouse convulsions.

Key words: citalopram, serotonin, MAO-A, Trp-OHase, El mouse

The El mouse was discovered in 1954, registered internationally as a mutant mouse in 1964 (1, 2) and established electroencephalographically as an authentic epilepsy model in 1976 (3). Seizures could be induced by vestibular stimuli, such as "tossing up" the mouse. The seizures generally appear with the onset of sexual maturity and persist throughout life (1). Manifestations of seizures in this strain may include limb and face automatisms such as chewing and salivation (2). Electrical discharges originate in deep limbic structures (3). Because of these features, the El mouse is thought to be an excellent animal model of human complex partial seizure or temporal lobe epilepsy (4-6).

Biochemical studies of El mice have suggested the presence of monoaminergic abnormalities in the brain (7, 8). Studies of serotonin (5-HT) metabolism using [^{14}C] 5-HT showed a higher rate of 5-HT synthesis in the brain of the El mouse during the interictal stage compared with that of the ddY mouse, which is the maternal strain of El mice. The monoamine oxidase (MAO) activity in the cerebellum of stimulated El (El_s) mice was higher than that of non-stimulated El (El_{ns}) mice (8), and the 5-HT receptor binding of [^{3}H] 5-HT in the cerebral cortex and brainstem in the interictal period was lower in El_s mice than in El_{ns} mice (8). On the other hand, we have also reported that El_s mice have lower levels of dopamine (DA), norepinephrine (NE), and 5-HT than El_{ns} mice during the interictal stage (9), and that convulsions were completely inhibited by significant elevation of cortical 5-HT levels in El_s mice injected with piperine (10). We also found that the levels of 5-HT and 5-hydroxyindolacetic acid (5-HIAA) increased with the susceptibility to seizures (11). These findings suggest that 5-HTergic nervous functions are closely related to the seizure susceptibility in El mice, and that the synaptic function of 5-HTergic nerve cells was inhibited in El mice, especially in the cerebral cortex.

On the other hand, citalopram (1-(3-dimethylaminopropyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile) is known as a specific 5-HT reuptake in-

* To whom correspondence should be addressed.

hibitor with an IC_{50} of 1.8nM (12), and is used as an anti-depressant in Europe (13, 14).

In the present study, we examined the effects of citalopram on El mice seizures, on the levels of 5-HT and 5-HIAA, and on the activity of MAO-A and tryptophan hydroxylase (Trp-OHase) in the El mouse brain.

Materials and Methods

Animals. El mice (20–25g, Central Institute for Experimental Animals, Japan) were used for this study. They were housed at 25°C with a 12-h light-dark cycle (6:00–18:00 lights on) and were allowed free access to water and a standard diet (MF, Oriental Yeast, Japan). From 8 weeks of age, they were given tossing-up stimulations once a week, and until 13 weeks of age all of them had seizures. Then the mice were divided into two groups: one group (n = 80) received citalopram per os and the other (n = 80) intraperitoneally.

Examination by per os administration. This group was further divided into two groups. One group (n = 40) was allowed free access to water and a standard diet for control, and the other group (n = 40) was given a diet containing citalopram 0.04 % (80mg/kg/day) for 2 weeks. Then, the anticonvulsant effect, 5-HT and 5-HIAA levels, and MAO-A and Trp-OHase activities were tested. Each examination was performed using separate animals.

Anticonvulsant effect of citalopram. The tossing up stimulation test was performed using 10 mice in each group. The number of tossing up stimulations required to induce convulsions was compared with that observed before citalopram or vehicle administration by the paired *t*-test.

5-HT and 5-HIAA levels. The mice were killed by exposing the head to microwave irradiation at 3 kW for 0.2 sec using a Metabostat NJE 2601 (New Japan Radio, Japan). The whole brain was removed, and the cerebral cortex, striatum, hippocampus, midbrain and hypothalamus were rapidly separated on an ice plate according to the method of Glowinski and Iversen (15). Samples were stored at −80°C until analysis. The brain tissue of each region was homogenized with 200mM ice-cold perchloric acid. After centrifugation (10,000 × *g*, for 30min at 4°C), the supernatant was filtered (0.45μm mesh) and then injected directly into the HPLC system. The HPLC system consisted of a delivery pump (Model L-4,000W, Yanagimoto, Kyoto, Japan), an analytical column (LiChrospher RP-18, 250mm × 4mm I.D., particle size 5μm, Cica-MERCK, Tokyo), and an electrochemical detector (Model EC-100, EICOM, Kyoto, Japan) with a glassy carbon electrode, which was used at 700mV *versus* an Ag/AgCl reference electrode. The mobile phase consisted of 100mM potassium phosphate buffer (pH 3.5) containing 20 % methanol, 150mg/l sodium octane-sulphonate (Nacalai Tesque Inc., Kyoto, Japan), and 10mg/l EDTA (Katayama Chemical, Osaka, Japan), and was pumped at 0.8ml/min. Protein contents were determined by the method of Lowry *et al.* (16).

MAO-A activity. The mice were killed by decapitation, and then the forebrain was removed and stored at −80°C until analysis. The brain tissue was homogenized with three volumes of HEPES buffer (50mM, pH 7.6) (Wako Pure Chemical Industries, Osaka, Japan). After centrifugation (25,000 × *g*, for 40min at 4°C), HEPES buffer (600mM, pH 7.6, 50μl), bovine serum albumin (15mg/l, 10μl) (Sigma Chemical, St. Louis, MO, USA), and H_2O (180μl), were added to supernatants (50μl). After preincubation for 5min at 37°C, 5-HT (130μM, 10μl) (Wako Pure Chemical Industries) or pargyline (20mM, 10μl) (Sigma Chemical) was added to the reaction mixtures and the incubation was continued for an additional 20min. The reaction was stopped by the addition of perchloric acid to the mixture. The samples were centrifuged (10,000 × *g*) for 30min at 4°C, filtered (0.45μm mesh) and then injected directly into the HPLC system as described above. The difference in the 5-HIAA levels between 5-HT-added and pargyline-added samples was taken as the MAO-A activity.

Trp-OHase activity. Trp-OHase activity was measured according to the method of Friedman *et al.* (17). The mice were killed by decapitation, and then the forebrain was removed and stored at −80°C until analysis. The brain tissue was homogenized with three volume of HEPES buffer (50mM, pH 7.6), and centrifuged (25,000 × *g*, for 40min at 4°C). The supernatant (100μl), to which was added HEPES buffer (600mM, pH 7.6, 50μl), L-Trp (10mM, 10μl) (Katayama Chemical Industries), dithiothreitol (50mM, 10μl) (Wako Pure Chemical Industries), ferrous ammonium sulfate (1mM, 10μl) (Wako Pure Chemical Industries), catalase (10mg/ml, 10μl) (Sigma Chemical), 6-methyl-5,6,7,8-tetrahydropterine ($6MPH_4$) (5mM, 10μl) (Calbiochem, LA, USA), pargyline (20mM, 10μl), 3-hydroxybenzylhydrazine (20mM, 10μl) (Katayama Chemical)

and H_2O (80 μl), was incubated for 20 min at 37°C. $6MPH_4$ was omitted to obtain the blank data. After incubation, perchloric acid was added to the medium, which was then centrifuged (3,000 × g) for 15 min at 4°C. Finally, HCl solution (5 N, 300 μl) was added to the supernatant (200 μl). The level of L-hydroxytryptophan together with 5-HT in the sample was measured using a photoelectric fluorophotometer (excitation 295 nm, emission 530 nm: Hitachi 650-10S, Fluorescence Spectrophotometer, Hitachi, Japan). The difference in the levels (with and without $6MPH_4$) was taken as the Trp-OHase activity.

Examination by intraperitoneal administration. This group was also further divided into two groups: In one group (n = 40) the mice were injected intraperitoneally with saline as a control, and in the other group (n = 40) they were injected with citalopram (80 mg/kg, i.p.). Two hours after injection, the anticonvulsant effect, 5-HT and 5-HIAA levels, and MAO-A and Trp-OHase activities were determined. Each examination was performed using different animals, and the methods used were the same as described above.

The differences in the 5-HT and 5-HIAA levels and MAO-A and Trp-OHase activities were tested for significance using one-way analysis of variance.

Results

Anticonvulsant Effect of Citalopram

Examination by per os administration. The number of tossing up stimulations (the seizure threshold) decreased with the experimental procedure in the control group, and increased in the citalopram administration group (80 mg/kg/day for 14 days) (Fig. 1). No convulsions occurred in three of the ten mice in the citalopram group.

Examination by intraperitoneal administration. There was no significant difference in the seizure thresholds before and after saline or citalopram administration (80 mg/kg, i.p.) (Fig. 2).

Effect of Citalopram on the Levels of 5-HT and 5-HIAA

The 5-HT and 5-HIAA levels in the per os control group were similar to those in the intraperitoneal control group. There was no significant difference in the 5-HT level among the per os citalopram group, the intraperitoneal citalopram group, and the control groups in any regions of the brain measured in this examination. The 5-HIAA level in both the per os and intraperitoneal citalopram groups decreased in all regions measured compared with the control groups. There was almost no difference between the per os and the intraperitoneal

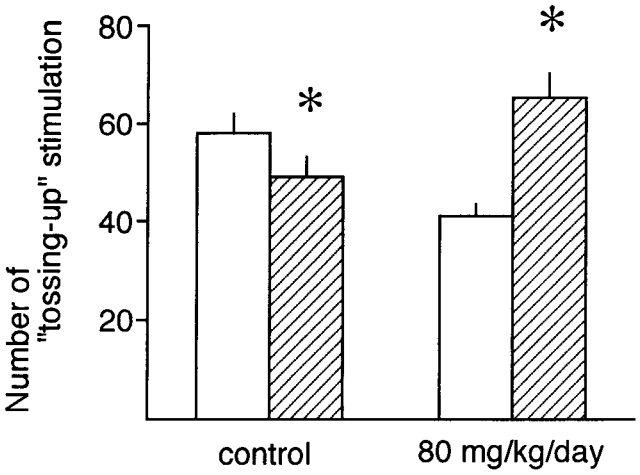

Fig. 1 Anticonvulsant effect of citalopram. Citalopram was administered per os for 14 days by addition to the standard diet (80 mg/kg/day). Open columns represent the mean ± SEM of the number of tossing-up stimulations (the seizure threshold) before the administration, and hatched columns represent that after the administration. *: $P < 0.05$ by paired t-test. n = 10

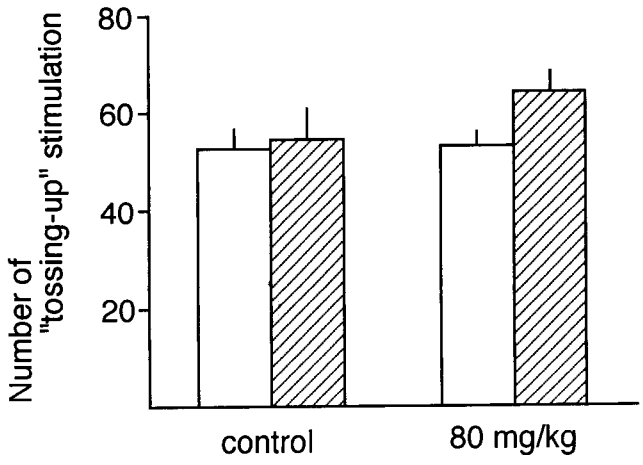

Fig. 2 Anticonvulsant effect of citalopram. Citalopram was administered intraperitoneally (80 mg/kg). Open columns represent the mean ± SEM of the number of tossing-up stimulation (the seizure threshold) before the administration, and hatched columns represent that 2 h after the administration. *: $P < 0.05$ by paired t-test. n = 10

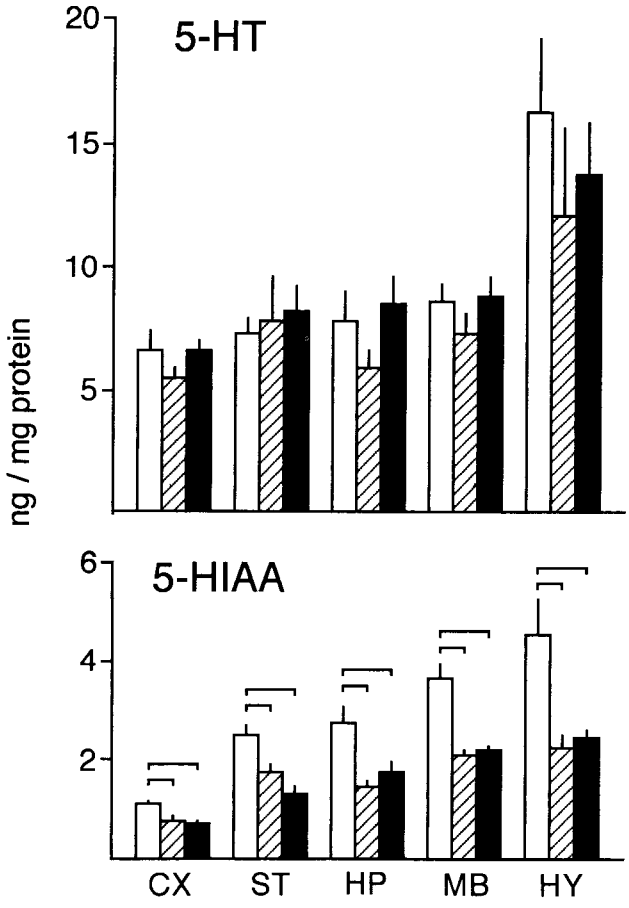

Fig. 3

citalopram groups (Fig. 3).

Effect of Citalopram on MAO-A and Trp-OHase Activities

There was no significant difference in Trp-OHase activity between the control groups and the citalopram groups. MAO-A activity, however, was significantly decreased after per os administration compared with the control groups, but citalopram administered intraperitoneally had no significant effect (Fig. 4).

Discussion

El mice are known to be very sensitive to stress, and emotional stress inhibits their convulsions (18). As saline injected intraperitoneally once a day to El mice inhibited the convulsions completely within 4 days, chronic

Fig. 3 The effect of citalopram on the levels of 5-HT and 5-HIAA. Open columns represent the control value (mean ± SEM, n = 20), hatched columns represent the per os value (mean ± SEM, n = 10) and closed columns represent the intraperitoneal value (mean ± SEM, n = 10). CX: cerebral cortex, ST: striatum, HP: hippocampus, MB: midbrain, HY: hypothalamus. ⌐¬: $P < 0.05$ by one-way analysis of variance (ANOVA).

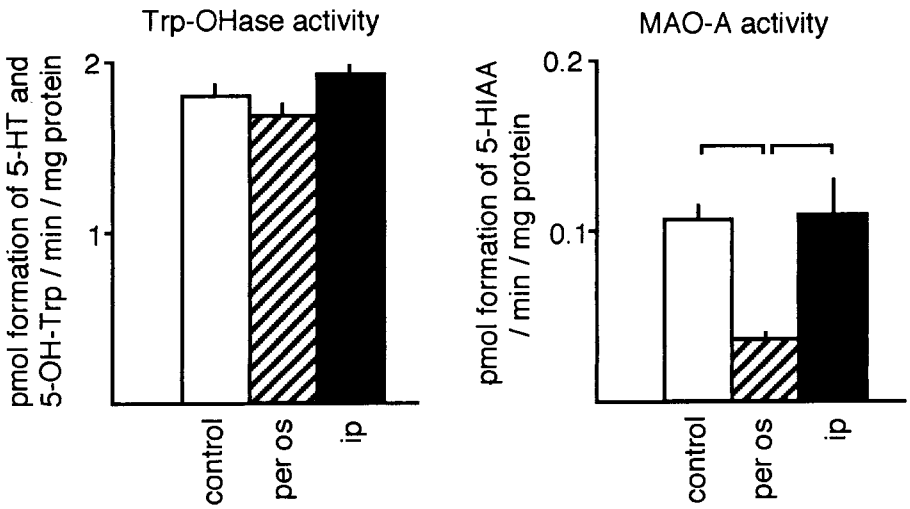

Fig. 4 The effect of citalopram on the activity of Trp-OHase and MAO-A. L-Trp concentration as substrate for Trp-OHase is 0.333 mM, and 5-HT for MAO-A is 4.35 μM. Symbols are the same as in Fig. 3.

intraperitoneal injection with saline was considered to be harsh treatment for El mice. Therefore, although we first tried administering citalopram chronically by intraperitoneal injection, we abandoned this route of administration. El mouse convulsions were also inhibited when the vehicle was administered using an orogastric tube.

Then citalopram was mixed in the standard diet at 0.04 %. When the mice were allowed free access to this diet, our preliminary data indicated that the daily intake of citalopram was about 80 mg/kg, and the concentration of citalopram in the plasma became 538 nM after 14 days of administration. The administration of citalopram per os for 1 day did not increase the threshold of El mice convulsions and did not change the 5-HT and 5-HIAA levels in the El mouse brain. So we chose the intraperitoneal route for the acute model and oral administration for the chronic model, and compared between a single intraperitoneal injection group (80 mg/kg) and an oral administration group (80 mg/kg/day, for 14 days).

The threshold of El mice convulsions was not increased by the single citalopram administration, but the convulsions were depressed almost completely by the chronic administration of citalopram for 14 days. In spite of these differences, similar changes in 5-HT and 5-HIAA levels were observed in the El mouse brain regardless of the route of administration. We also observed no change in the activity of Trp-OHase after citalopram administration. The activity of MAO-A, which catabolizes 5-HT, was decreased by chronic administration of citalopram. 5-HT is metabolized to 5-HIAA in the mitochondria after the reuptake into the presynaptic cells, and citalopram inhibits this reuptake. Chronic administration of citalopram induces a continual reduction of 5-HT supply to the mitochondria. This continual reduction of MAO-A substrate may decrease MAO-A activity. We observed that the chronic administration of citalopram decreased not only 5-HT metabolism but also metabolism (19). Trp is known to be metabolized by two main pathways in the mammalian brain: One is the production of 5-HT, and the other is production of kynurenine (KYN) via xanthurenic acid through formylkynurenine. KYN is reported to be an endogenous excitant in the central nervous system (20, 21). Nishijima reported that 3-hydroxyanthranilic acid and *o*-aminophenol, both of which are KYN metabolites, induced spike discharges in rat electrocorticograms (22). KYN and its metabolites are closely related to convulsive mechanisms. Furthermore, we found that El mice had a higher level of KYN in the brain compared with ddY mice (23), suggesting that KYN may be involved in the seizure mechanism. Acute administration of citalopram may inhibit 5-HT reuptake, while chronic administration may also inhibit Trp metabolism by both the 5-HT and KYN pathways and decrease KYN. Hiramatsu reported that El mouse seizures were inhibited by 5-HTergic neuronal activity (7). In this study, citalopram inhibited convulsions in the El mouse by chronic administration, but not by acute administration. The level of 5-HT in the brain did not change, and the level of 5-HIAA decreased in both citalopram groups. The changes in 5-HT and 5-HIAA levels were the same for acute and chronic administration. The administration of citalopram for 14 days decreased MAO-A activity probably by decreasing the amount of substrate present. These findings suggest two possibilities: One is that the continual activation of 5-HTergic neurons is necessary to inhibit seizures in El mice; the other is that the 5-HTergic neuron is not closely related to the development of El mouse seizures, and that chronic administration of citalopram may inhibit El mice seizures by affecting another neuronal system in addition to 5-HTergic neurons.

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