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Rapid Communication

Status Epilepticus Decreases Brain Cytochrome P450 2D4 Expression in Rats

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ABSTRACT

Status epilepticus (SE) is a life-threatening neurological emergency characterized by frequent seizures. The present study aims at elucidating the effect of SE on CYP2D4 expression in the rat brain. To create a rat model of SE, Sprague-Dawley rats were intraperitoneally administered 10 mg/kg kainic acid. The CYP2D4 mRNA levels in the cortex and hippocampus of the SE rats were decreased by 0.38- and 0.39-fold, respectively. The protein level of octamer transcription factor 1 (Oct-1), which is involved in the transcriptional activation of CYP2D4 by binding to the *CYP2D4* regulatory element, was also attenuated by 0.64- and 0.51-fold in these regions of the SE rat, suggesting that a reduction in Oct-1 may be involved in the CYP2D4 suppression. Yin yang 1 can function as a cofactor of histone deacetylase 1 and inhibit the binding of Oct-1 to the *CYP2D4* regulatory element. The coimmunoprecipitation assay revealed that the interaction between yin yang 1 and histone deacetylase 1 in the cortex and hippocampus was enhanced during SE, indicating that this interaction is also responsible for the CYP2D4 suppression. This study clarified that SE led to a decrease in the expression of CYP2D4, thus altering the pharmacokinetics and efficacy of the drugs in the brain.

Introduction

Epilepsy is a common neurological disorder characterized by seizures that affects people in every country throughout the world.[1] Frequent epileptic seizures without recovery of consciousness are termed status epilepticus (SE). This is a dangerous condition that leads to neural damage or death.[2] It is also characterized by brain inflammation. It is believed that propagation of the epileptiform activity from the CA3 region of the hippocampus to the cortex[3] causes the neural inflammation in the hippocampus and cortex.[1]

For studies on SE, rat models, which are generated by intraperitoneal administration of kainic acid (KA), a kainate receptor agonist, are commonly used because they exhibit neural inflammations similar to that caused by SE in humans.[1] The kainate receptor is highly expressed in the CA3 region of the hippocampus.[4]

CYP2D6 is one of the most important CYP isoforms in the human liver, which is involved in the metabolism of many central-acting drugs.[5] Recent study has been reported that human CYP2D6 is detected in the various brain regions.[6] In the clinical settings, maintaining a drug concentration at the appropriate level in the target site (a specific brain region or neurons) is important for performing effective and safe drug therapy. Nicotine increases codeine analgesia through the induction of CYP2D and activation of codeine to morphine in the brain, but not in the liver,[7] suggesting that the changes in the expression level of brain CYP2D may affect the efficacy and pharmacokinetics of central-acting drugs in the brain. In the rat brain, CYP2D4 is one of the major CYP isoforms,[6,8] and its gene sequence has a 77% similarity with human CYP2D6.[9] It has been reported that the expression of brain CYP2D is reduced by inflammation caused by lipopolysaccharide injection into the lateral ventricle.[10] However, whether the SE-induced inflammation altered the CYP2D4 expression has not been clarified.

This study aims to elucidate the effect of SE on CYP2D4 expression in the rat brain. Furthermore, we investigated whether the mechanisms of the CYP2D4 alteration by SE were mediated by octamer transcription factor 1 (Oct-1), yin yang 1 (YY1), and histone deacetylase (HDAC) 1.

Materials and Methods

Evaluation of KA-Induced Seizures in Rats

All experiments were approved by the Institutional Animal Care and Use Committee of Meijo University. Seven-week-old male

Abbreviations used: HDAC, histone deacetylase; KA, kainic acid; Oct-1, octamer transcription factor 1; SE, status epilepticus; YY1, yin yang 1.

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Sprague-Dawley rats (Japan SLC, Hamamatsu, Japan) were either treated with KA (10 mg/kg, intraperitoneally, $n = 10$; Abcam, Cambridge, MA) or with saline, as the control (CT rats, $n = 5$). After the treatment with KA, the rats were observed for behavioral changes over a period of 2.5 h. The intensity of the seizure response was scored in accordance with the classification by Morales-Garcia et al[11] as follows: stage 0, nonresponse (NR; $n = 4$); stage 1, facial movements ($n = 1$); stage 2, head nodding ($n = 0$); stage 3, forelimbs clonus ($n = 0$); stage 4, rearing ($n = 0$); and stage 5, rearing and falling ($n = 5$). SE was defined as a continuous stage 4 or 5 seizure lasting more than 10 min. The rats exhibiting SE (SE rats) and NR (NR rats) were used in the present study. The cerebellum, cortex, and hippocampus were collected at 24 h after KA administration.

Measurement of CYP2D4 mRNA Expression by Real-Time Polymerase Chain Reaction

To investigate the effects of SE on changes in CYP2D4 mRNA in rats, the CT ($n = 5$), SE ($n = 5$), and NR ($n = 4$) rats were used. Total RNA was isolated from the brain regions using the TRIzol reagent (Life Technologies, Carlsbad, CA). Complementary DNA was synthesized using ReverTra Ace qPCR Kit (TOYOBO, Osaka, Japan). The CYP2D4 mRNA levels were measured using the TaqMan gene expression assay (Rn01504629_m1; Applied Biosystems, Foster City, CA). The data were normalized to the data on β-actin.

Dextromethorphan O-demethylation

Pooled brain microsomes from the cerebellum, cortex, and hippocampus of the CT ($n = 5$), SE ($n = 5$), and NR ($n = 4$) rats were prepared as described previously.[12] The dextromethorphan *O*-demethylation in the rat brain was determined according to a previously described method[13] with slight modifications. In the present study, dextromethorphan *O*-demethylation was measured at 50-μM dextromethorphan because the S_{50} value for recombinant rat CYP2D4 was reported to be 43.2 ± 7.56 μM.[14] The incubation mixture contained 100-mM phosphate buffer (pH 7.4), 50-μM dextromethorphan, 0.5 mg/mL microsomal protein, and β-nicotinamide adenine dinucleotide phosphate–generating system (final concentration, 2.8-mM β-nicotinamide adenine dinucleotide phosphate oxidized form, 10-mM $MgCl_2$, 50-mM glucose-6-phosphate, and 10 U glucose-6-phosphate dehydrogenase). The reaction mixtures were incubated at 37°C for 60 min. Dextrorphan was quantified by liquid chromatography–tandem mass spectrometry. The detection limit for dextrorphan was 2.0 fmol. The quantification limit in the reaction mixture was 0.2 nM with a coefficient of variation less than 10%. In a preliminary study, the incubation time and protein concentrations were found to be in the linear range.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis/ Western Blot Analysis

The nuclear extracts were prepared from cerebellum, cortex, and hippocampus following a previously described method,[15] and the samples from 5 rats of the CT and SE, respectively, were pooled. The extracts were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and immunodetection was performed using the specific primary antibodies, which were mouse anti-Oct-1 (sc-8024; Santa Cruz Biotechnology, Santa Cruz, CA) at a 1:200 dilution, mouse anti-YY1 (sc-7341; Santa Cruz Biotechnology, 1:200 dilution), mouse anti-HDAC1 (sc-81598; Santa Cruz Biotechnology, 1:200 dilution), and rabbit anti-lamin B1 antibody (ab16048; Abcam, 1:1000 dilution).

Coimmunoprecipitation Assay

For coimmunoprecipitation assays, 3000 μg of nuclear extracts were incubated with 5 μg of rabbit anti-HDAC1 (ab7028; Abcam) or normal rabbit IgG antibodies (PM035; MBL Science, Nagoya, Japan) at 4°C for 18 h. Next, protein G plus-agarose beads (sc-2002; Santa Cruz Biotechnology) were added and incubated at 4°C for 2 h. After washing the beads 3 times with radioimmunoprecipitation buffer, sodium dodecyl sulfate sample buffer was added and boiled at 100°C for 10 min. The mixture was centrifuged at 10,000 *g* for 5 min, and the supernatant was used for Western blot analysis.

Statistical Analysis

Statistical analysis of the experimental data was performed using the KaleidaGraph computer system (Synergy Software, Reading, PA). The comparison of multiple groups was carried out using 1-way ANOVA followed by Tukey's post hoc tests. Student paired t-test was used for comparison within 2 groups.

Results and Discussion

The expression of CYP2D4 mRNA in the SE rats was decreased by 0.38- and 0.39-fold in the cortex and hippocampus, respectively, compared to the CT rats (Fig. 1); however, it was not decreased in the cerebellum. On the other hand, the CYP2D4 mRNA expression in the NR rats was not reduced in the cortex and hippocampus, respectively, compared to the CT rats, suggesting that SE, not KA administration, attenuated the expression of CYP2D4. The levels of inflammatory cytokines, such as interleukin-1β and tumor necrosis factor-α, were increased in the hippocampus and cortex of the rats with KA-induced SE but not in the cerebellum.[16] Thus, it is suggested that the reduction of CYP2D4 in the brain during SE is related to seizure development. Dextromethorphan *O*-demethylation in the SE rats was attenuated by 0.43- and 0.47-fold in the cortex and hippocampus, respectively, as compared to the CT rats (Fig. 2); however, it was unchanged in the cerebellum. Because

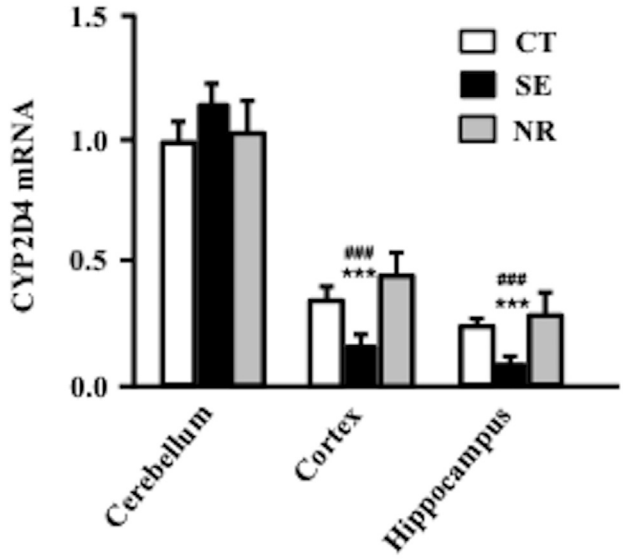

Figure 1. Decrease in the expression of brain CYP2D4 mRNA in the SE rats. The expression level of CYP2D4 mRNA was calculated relative to that in the cerebellum of CT rats and was normalized to the level of β-actin. Each column represents the mean ± SD (CT, $n = 5$; SE, $n = 5$; NR, $n = 4$). $^{***}p < 0.001$ compared with the corresponding CT rats. $^{###}p < 0.001$ compared with the corresponding NR rats.