Curcumin inhibits amygdaloid kindled seizures in rats
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Background Curcumin can reduce the severity of seizures induced by kainate acid (KA), but the role of curcumin in amygdaloid kindled models is still unknown. This study aimed to explore the effect of curcumin on the development of kindling in amygdaloid kindled rats.

Methods With an amygdaloid kindled Sprague-Dawley (SD) rat model and an electrophysiological method, different doses of curcumin (10 mg·kg⁻¹·d⁻¹ and 30 mg·kg⁻¹·d⁻¹ as low dose groups, 100 mg·kg⁻¹·d⁻¹ and 300 mg·kg⁻¹·d⁻¹ as high dose groups) were administrated intraperitoneally during the whole kindling days, by comparison with the course of kindling, afterdischarge (AD) thresholds and the number of ADs to reach the stages of class I to V seizures in the rats between control and experimental groups. One-way or two-way ANOVA and Fisher's least significant difference post hoc test were used for statistical analyses.

Results Curcumin (both 100 mg·kg⁻¹·d⁻¹ and 300 mg·kg⁻¹·d⁻¹) significantly inhibited the behavioral seizure development in the (19.80±2.25) and (21.70±2.21) stimulations respectively required to reach the kindled state. Rats treated with 100 mg·kg⁻¹·d⁻¹ curcumin 30 minutes before kindling stimulation showed an obvious increase in the stimulation current intensity required to evoke AD from (703.3±85.9) µA to (960.0±116.5) µA during the progression to class V seizures. Rats treated with 300 mg·kg⁻¹·d⁻¹ curcumin showed a significant increase in the stimulation current intensity required to evoke AD from (735.0±65.2) µA to (867.0±93.4) µA during the progression to class V seizures. Rats treated with 300 mg·kg⁻¹·d⁻¹ curcumin required much more evoked ADs to reach the stage of class both IV (as (199.83±12.47) seconds) and V seizures (as (210.66±10.68) seconds). Rats treated with 100 mg·kg⁻¹·d⁻¹ curcumin required much more evoked ADs to reach the stage of class V seizures (as (219.56±18.24) seconds).

Conclusion Our study suggests that curcumin has a potent antiepileptogenic effect on kindling-induced epileptogenesis.

Curcumin, the yellow, bioactive component of turmeric, the powdered rhizome of *Curcuma longa* *Linn*, is well known to be a potent antioxidant in various *in vivo* and *in vitro* models. Recent studies also demonstrate the chemopreventive properties of this compound and its ability to inhibit various signaling cascades and transcriptional activities. There is increasing interest in investigating if curcumin offers neuroprotection against oxidative stress resulting from different forms of brain injury, even though studies to elucidate its mechanisms of action in the central nervous system (CNS) have been inconclusive. Curcumin has been shown to ameliorate Alzheimer's disease pathology and neuronal damage resulting from cerebral ischemia, chronic ethanol exposure, 6-hydroxydopamine (6-OHDA) model of Parkinson's disease. Some research groups have reported that curcumin administration in fully kindled rats induced by KA can prevent the neuronal death and reduce the level of seizure. Recently some research indicates that curcumin can reduce the severity of seizure through affecting the histone modification of chromatin. Taken together, these experiments suggest the potent anti-epileptic effect of curcumin in the epilepsy model.

Curcumin is reported to have unexpected effects on CNS, including affecting phosphorylation of N-methyl-D-aspartic acid (NMDA) receptors and expression of brain-derived neurotrophic factor (BDNF), which indicates that curcumin may exert its function through affecting the neuronal excitability. Our recent research indicates that curcumin could inhibit calcium elevation induced by KA in primary cultured hippocampal neurons. The question of whether curcumin can affect epilepsy development using the amygdaloid kindled rat model is of great interest.

METHODS

Animals A total of 55 adult male Sprague-Dawley (SD) rats were used in this study. This study was supported by grants from National Natural Science Committee (No.30470593), a fund from Institute of Brain Science of Fudan University (2008), and a youth fund from Shanghai Municipal Health Bureau (2009).
(200–250 g, about 2.5 months old) were bought from Shanghai SLAC Animal Co., Ltd. (China) and maintained on a 12-hour light/dark cycle with ad libitum access to food and water. Procedures involving animals and their care were performed in accordance with the Animal Care and Use Committee of the Institute of Neuroscience, Chinese Academy of Science. All efforts were made to minimize the number of animals used and their suffering.

**Electrode implantation**
Electrodes were implanted in all rats used for this study. Under chloral hydrate anesthesia (250 mg/kg, i.p.), a bipolar electrode, which consisted of two twisted teflon-coated stainless steel wires (diameter, 250 µm) used for stimulation and recording, was stereotaxically implanted in the left amygdala (2.8 mm posterior to the bregma, 4.9 mm lateral to the midline, and 8.6 mm below the dura). Two screws were inserted into the skull through a drilled hole without piercing the dura. One of the screws (0.8 mm anterior to the bregma, 3.0 mm right lateral to the midline) served as ground electrode; another served as reference (6.0 mm posterior to the bregma, 3.0 mm right lateral to the midline) in the electroencephalographic (EEG) recording. After surgery, the rats were treated with antibiotics for 1 week to prevent infection.

**Drug administration and kindling procedure**
Of the 55 electrode-implanted rats, 4 rats could not be included in the experiments because of death during anesthesia or misplacement of the electrode. The remaining 51 rats were randomly assigned to the following treatment groups: group 1, vehicle control (n=10); group 2, curcumin 10 mg·kg⁻¹·d⁻¹ (n=10); group 3, curcumin 30 mg·kg⁻¹·d⁻¹ (n=11); group 4, curcumin 100 mg·kg⁻¹·d⁻¹ (n=10); and group 5, curcumin 300 mg·kg⁻¹·d⁻¹ (n=10).

An identical volume of curcumin (Sigma, St Louis, MO, USA) and vehicle controls were used for injection (3 ml). Doses and pretreatment time of curcumin were chosen based on its previous studies in different cerebral injury models in rodents, including the epilepsy model in rats.

After 1 week for postoperative recovery, the EEG seizure threshold was determined by application of a 1 second train of 1 millisecond monophasic rectangular pulses at 62 Hz beginning at 50 µA. The 25 µA steps were administered at 2 minutes interval until an afterdischarge (AD) lasting for at least 5 seconds was detected. Animals whose stimulation intensity was larger than 400 µA were excluded. The intensity of AD threshold plus 100 µA was administered once a day 30 minutes after administration of vehicle or curcumin (10, 30, 100 and 300 mg·kg⁻¹·d⁻¹, i.p.) dissolved as described above in the afternoon five days a week for the next 21 days. Rats were not kindled or treated over the weekends.

With each stimulation, seizure severity was scored according to the study of Racine by an observer blinded to the treatment conditions: I, immobility, eye closure, ear twitching, twitching of vibrissae, sniffing, facial clonus; II, head nodding associated with more severe facial clonus; III, clonus of one forelimb or bilateral clonus without rearing; IV, bilateral clonus accompanied by rearing or generalized clonic seizures without rearing and falling; and V, rearing and falling accompanied by generalized clonic seizures. Stimulations were terminated when all rats of the vehicle treated group had reached kindling criterion (3 consecutive class V seizures).

During the kindling process, total AD duration (ADD) was recorded in addition to seizure severity. ADD was defined as the period of high amplitude spiking (at least 1 Hz frequency and twice the prestimulation amplitude) in the EEG of the electrode, including the time of stimulation.

**Statistical analysis**
All statistical analyses were performed using SPSS (Cary, NC, USA) 13.0 software. Values were expressed as mean ± standard error (SE). The data among the groups were compared using one-way or two-way analysis of variance (ANOVA). Between-groups variance was determined by a Fisher’s least significant difference post hoc test after ANOVA. Statistical significance was defined as P <0.05.

**RESULTS**

Curcumin suppressed the behavioral progression of amygdaloid kindled rats
Compared with rats treated with vehicle and 10 mg/kg, 30 mg/kg curcumin, both 100 mg/kg and 300 mg/kg curcumin suppressed the behavioral progression of kindling (Figure 1). Injection of both 100 mg/kg and 300 mg/kg curcumin inhibited the behavioral seizure development as evident in the (19.80±2.25) and (21.70±2.21) stimulations respectively required to reach the kindled state, compared with (16.10±1.66) stimulations for vehicle-treated animals (Figure 2).

Curcumin increased the AD thresholds in amygdaloid kindled rats
Rats in the control group showed a gradual reduction in AD threshold from (755.7±19.6) µA at the initiation of kindling stimulation to (545.9±32.6) µA after repeated stimulations to the stage of class V seizures, consistent with the progressive features of kindling. In experimental groups, rats treated with 100 mg/kg curcumin 30 minutes before kindling stimulation showed an increase in the stimulation current intensity required to evoke AD from (703.3±85.9) µA to (960.0±116.5) µA during the progression to class V seizures. In addition, rats treated with 300 mg/kg curcumin also showed an increase in the stimulation current intensity required to evoke AD from (735.0±65.2) µA to (867.0±93.4) µA during the progression to class V seizures. The effects of curcumin on AD threshold normalized to the initial AD before treatment with saline or curcumin were plotted as a function of AD number (Figure 3).
**Figure 1.** Effect of curcumin on acquisition of kindling upon daily stimulation of the basolateral amygdala in rats. A–D show data for vehicle (n=10) and curcumin 10 mg·kg⁻¹·d⁻¹ (n=10), 30 mg·kg⁻¹·d⁻¹ (n=11), 100 mg·kg⁻¹·d⁻¹ (n=10) and 300 mg·kg⁻¹·d⁻¹ (n=10), respectively. Data are shown for each stimulation day. There are significant differences between controls and curcumin for the 100 mg·kg⁻¹·d⁻¹ group (P<0.05) and the 300 mg·kg⁻¹·d⁻¹ group (P<0.05) but not the 10 mg·kg⁻¹·d⁻¹ group and the 30 mg·kg⁻¹·d⁻¹ group.

**Curcumin evoked more AD to reach the stage of class IV or V seizures**

The cumulative ADD to reach class II–V was calculated respectively by summing individual ADD until the first class II–V seizure was reached. Rats treated with 300 mg/kg curcumin required more evoked ADs to reach the stage of class both IV (as (199.83±12.47) seconds) and V (as (210.66±10.68) seconds) seizures. Rats treated with 100 mg/kg curcumin required more evoked ADs to reach the stage of class V (as (219.56±18.24) seconds) seizures (Figure 4).

**DISCUSSION**

The purpose of this study was to explore the effect of curcumin on the development of amygdaloid kindling in rats. The results indicated that systemic administration of higher doses of curcumin (both 100 mg·kg⁻¹·d⁻¹ and 300 mg·kg⁻¹·d⁻¹) could suppress the progression of kindling.

It has been reported that intraperitoneal administration of generalized seizures in KA treated mice. Intraperitoneal administration of 300 mg/kg curcumin suppressed the death of hippocampal neurons induced by generalized seizures in pilocapine-kindled rats. We chose different doses of curcumin as a high dose (100 mg/kg and 300 mg/kg) and a low dose (10 mg/kg and 30 mg/kg) in this study. Kinetic and bioavailability research indicated that the level of curcumin in the blood and brain peaked at 30–60 minutes after intraperitoneal administration of curcumin, so electrostimulation of rats began 30 minutes after curcumin administration.

The present data on kindling acquisition following stimulation of the basolateral amygdala show that curcumin significantly retards kindling at a high daily dose (100 mg/kg and 300 mg/kg), whereas a low dose (10 mg/kg and 30 mg/kg) was ineffective in this regard. At 100 mg/kg, the average numbers of stimulations needed to reach kindled class V was increased. There was also an increase in the cumulative ADDs needed to reach class IV and V seizures, but the former did not quite reach the level...
of statistical significance. At 300 mg/kg, the average numbers of stimulations to reach kindled class V was increased. There was also a significant increase in the cumulative ADDs needed to reach class IV and V seizures.

Based on the present data, curcumin (both 100 mg/kg and 300 mg/kg) has the potential to retard kindling-induced epileptogenesis. These data combined with the former researches indicate that curcumin did not simply mask the expression of kindled seizures through an anticonvulsant action, but could exert an antiepileptogenic effect.

The precise mode of action by which curcumin exerts its effects is incompletely understood. It has been suggested that curcumin can affect the phosphorylation of the 30 mg/kg curcumin can reduce the severity of secondarily NMDA subtype of glutamate receptors, but these results do not suggest a specific molecular mechanism.

In addition, curcumin is very affluent, inexpensive and relatively safe in human. It is administered in human weighing 70 kg at an approximate dose of 8 g/d (which is equivalent to approximately a dose of 640 mg in adult rats taking into account differences in surface areas and weights between two species) for 3 months without noticeable side effects.

The present data indicate that curcumin has a potent antiepileptic effect and demonstrate that this drug, in addition to exerting anticonvulsant activity, has the potential to retard kindling-induced epileptogenesis.

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REFERENCES