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Effects of curcumin on hippocampal neural activity in rats

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Curcuma rhizome, belonging to the Zingiberaceae family, is the root of *Curcuma longa* plant. Curcuma has a long history of use in traditional medicine of China and India, and it is used as a curry spice in food preparation [33]. Of the three main curcuminoids found in the turmeric root, curcumin is the most abundant and the most biologically active. Curcumin (C21H20O6) is the main pharmacologically active ingredient of the *Curcuma rhizome*. It is safe, with little to no toxicity, and possesses antitumor [27], antioxidant [30, 2], anti-inflammatory [4, 15], antiapoptosis, and lipid-reducing pharmacological effects [19]. As an anticarcinogen, an important target of curcumin is the Keap1 protein, which normally binds and sequesters Nrf2 in the cytoplasm. Curcumin can directly act on Keap1 to release Nrf2, which then translocates to the nucleus, where it heterodimerizes with small Maf proteins and binds to antioxidant response elements, inducing the expression of a large number of cytoprotective genes [11]. It was shown that curcumin can attenuate diabetes-induced apoptosis in retinal neurons by reducing the glutamate level and downregulating calcium/calmodulin-dependent protein kinase I [14].

There are literature data stating that curcumin significantly prevented the stress-induced decrease in 5-HT (1A) mRNA and BDNF (brain-derived neurotrophic factor) protein levels in the hippocampal subfields, two molecules involved in hippocampal neurogenesis [34]. Recent studies have shown that curcumin inhibits β-secretase and acetylcholinesterase in Aβ-induced animal model of Alzheimer's disease [7]. Curcumin has been reported to inhibit the activity of a variety of signaling enzymes in cells, such as NF-κB, mitogen-activated protein kinases, cyclooxygenase-1, Bcl-2, Bcl-xL and cyclin D1, that contribute to cellular survival and proliferation [1]. In Parkinson's disease rat model curcumin increased the contents of monoaminergic neurotransmitters, such as dopamine and norepinephrine [37].

Animal studies, in which researchers simulated depression by exercising animals to the point of exhaustion, have indicated that these remedies may work. They have shown that curcumin inhibits the enzyme monoamine oxidase

in the brain. Monoamine oxidase neutralises neurotransmitters, and administration of curcumin therefore boosts the concentration of serotonin, dopamine and noradrenalin in the brain. The neurochemical assays have shown that curcumin produces a marked increase of serotonin and noradrenaline levels at 10 mg/kg in both the frontal cortex and hippocampus [35].

In the animal study it was shown that doses of 10 and 20 mg curcumin per kg per day resulted in hippocampus growth by boosting the synthesis of the neural growth hormone BDNF [34]. An increase in BDNF synthesis is the result of the enzyme extracellular signal-related kinase being activated in brain cells [39]. Curcumin has been shown to have the ability to block or reverse the stress-induced changes typical of hypothalamic–pituitary–adrenal axis dysfunction to a level comparable to a typical tricyclic antidepressant including increases in corticosterone levels, reduced glucocorticoid receptor mRNA expression, and reduced levels of phosphorylated CREB (cAMP response element-binding protein) [36].

Dopamine is a special neurotransmitter because it is considered to be both excitatory and inhibitory. The origin of the dopaminergic afferents to the rat hippocampal formation has been investigated by measuring dopamine and DOPAC (3, 4-dihydroxyphenylacetic acid) contents in this area after electrolytic or chemical lesion of the ventral tegmental area (A10) or substantia nigra (A9). The dopaminergic afferents to the hippocampal formation originate from the A10 and A9 dopaminergic cell groups. The *anterior* hippocampal formation receives a major input from the A10 area whereas the *posterior* hippocampal region receives dopaminergic afferents from both A9 and A10 cell groups. The dopaminergic afferents are entering the hippocampal region mainly through the dorsal route [24]. Several lines of evidence suggest that dopaminergic (DA) neurotransmission influences hippocampal plasticity [13, 23] and function [22], but some recent works question whether these effects arise from direct dopaminergic afferents or from co-release of DA from noradrenergic fibers within the hippocampus [28]. There have been a number of studies supporting the functional role of dopaminergic D1/D5 receptors that are expressed in the hippocampus. Immunohistochemistry has revealed that these receptors are expressed in the dentate gyrus, CA1, and CA3 of the hippocampal formation [20], and on excitatory neurons [12]. DA enhances the excitability of hippocampal neurons by decreasing the calcium activated potassium conductance, thereby reducing the after hyper polarization of the action potential [16]. Further, inhibition of D1-type receptors prevented nicotine-induced in vivo synaptic potentiation measured in the dentate gyrus [31]. In addition, the amplitude of the dendritic action potential is enhanced by D1/D5 neurotransmission [9]. Recordings from hippocampal brain slices showed that D1/ D5 receptor neurotransmission supports induction of late phase long term potentiation in CA1 synapses, an effect that is dependent upon protein synthesis [10]. Furthermore, it was shown that D1/D5 neurotransmission is important for

the consolidation or persistence of long term memories [3]. Some previous studies have indicated that a larger proportion of norepinephrine terminals are located in the CA3 and dentate gyrus [18].

In the course of the current study the modulatory effects of curcumin on rats' hippocampal neuronal activity have been demonstrated under high frequency stimulation of ipsilateral entorhinal cortex.

Materials and Methods

Adult male albino rats (n=4) weighing 200±20 g were purchased from the experimental center of Orbeli Institute of Physiology. The animals were maintained at 25±2 °C and 12 h light – dark cycle, lights on 07:00 – 19:00 h. The animals were provided food and water ad libitum. All of the experimental protocols were approved by the Committee of Ethics of the Yerevan State Medical University (YSMU) (Yerevan, Armenia), followed the "Principles of laboratory animal care" and were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Reagents

Curcumin was purchased from Sigma-Aldrich. Curcumin (200 mg/kg) was dissolved in dimethyl sulfoxide (DMSO, 1 ml/kg).

In vivo electrophysiology and data analysis

In acute experiment the animals were anesthetized (Urethan 1.2 g/kg), immobilized with 1 % ditiline (25 mg/kg i/p), fixed in a stereotaxic head frame and were transferred to artificial respiration. The sample of isolated rat brain was obtained by transection of spinal cord (T2 – T3). The stimulating electrode was inserted into the ipsilateral entorhinal cortex (EC) according to stereotaxic coordinates [21] (AP – 9, L ±3.5, DV +4.0 mm) and a glass recording electrode (1–2 μm tip diameter) filled with 2 M NaCl was inserted into the hippocampal field at coordinates (AP−3.2-3.5; L±1.5-3.5; DV +2.8–4.0 mm) for recording spike activity flow of single neuron. High frequency stimulation (HFS) (100 Hz during 1 sec) was performed by means of rectangle pulses of 0.05 ms duration and 0.08-0.16 mA amplitude. Recording and mathematical analysis of spike activity were carried out on the basis of the program (worked by V.S Kamenetski) providing selection of spikes by amplitude discrimination, which pinpoints spikes and excludes artifacts during HFS, allowing not only post-tetanic, but also tetanic activity evaluation [38]. For statistical evaluation we used t-criteria of Student's t -test, the reliability of differences of interspike intervals before, after and during HFS. To increase reliability of statistical evaluations, we also used the non-parametric method of verification by application of Wilcoxon two-sample test, taking into account the asymptotic normality of this criterion and allowing comparison of the calculated values

with the table values of the standard normal distribution (at the significance levels 0.05, 0.01, and 0.001).

Results and Discussion

In single neurons of hippocampus treated with intraperitoneal (i.p.) injection of curcumine (200 mg/kg, once) (n=4), recording of background and evoked spike activities was carried out in the dynamics (from 1 to 63, 115 minutes) after curcumine exposure (after 5 minutes). The main effects lasted up to 30 and 80 minutes. The analysis revealed inhibitory (Fig. 1a, Fig. 2 a) and excitatory (Fig. 1 b, Fig. 2 a, b) effects of curcumin and restoring of the initial frequency of spike activity. The effects of single injection of curcumin are demonstrated in detail in the form of spike activity, expanded in real time (from -20 to 0 seconds) and after (0 to 20 sec) to HFS (High Frequency Stimulation) of entorhinal cortex: obviously slowing of pre- and post-stimulus frequency of neuron spiking 5-60, 71-99 minutes (Fig. 1a, Fig. 2b) and after curcumin administration in comparision with the initial rate (noted 0 min). Thus, in acutue electrophysiological experimets, in hippocampal neurons the inhibitory and excitatory effects were identified with a single intraperitoneal injection of curcumin (200 mg / kg, once), but it should be noted the characteristic fluctuation / oscillation in spike activity during the manifestation biased mix effects and variability of the beginning and the end of the mentioned effect, unlike persistent and reproducible effects (Fig. 2, 13-41 min, a, 16-38 min, b) of curcumin (200 mg / kg, i.p.). Curcumin effects on electrophysiological mechanisms such as synaptic plasticity are still unclear. In vitro studies in rat cerebral cortical neurons [29, 32] reported that curcumin has a concentration- and time-dependent neuroprotective effect against glutamate toxicity. In another study, curcumin attenuated glutamate levels and concentrations in the hippocampus and increased GABA and dopamine levels in several brain regions.

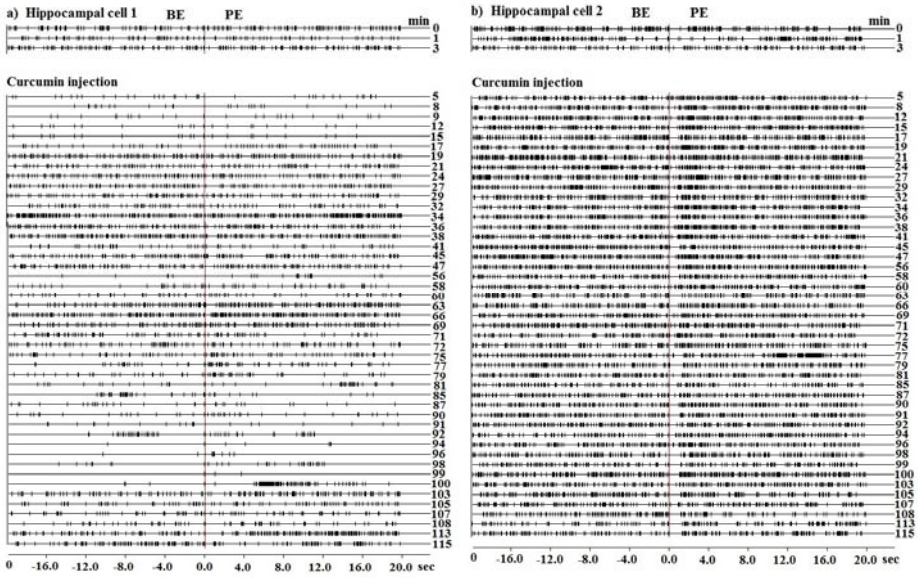

Figure 1. The picture of neuronal spike activity expanded in real time (40 sec), in the initial state (0 sec). In the right side it is shown the minute at which the activity was recorded after administration of curcumin; below – from 20 to 20 recording time (sec). Before stimulation (BE – before event) and post stimulation (PE – post event) spike activity of single neurons of hippocampus in dynamics of 0-115 min after administration of curcumin (200 mg/kg, i.p.)

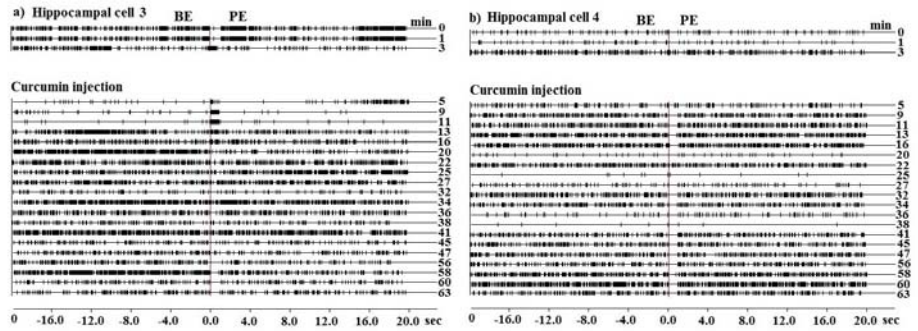

Figure 2. The picture of neuronal spike activity expanded in real time (40 sec), in the initial state (0 sec). In the right side it is shown the minute at which the activity was recorded after administration of curcumin; below – from 20 to 20 recording time (sec). Before stimulation (BE – before event) and post stimulation (PE – post event) spike activity of single neurons of hippocampus in dynamics of 0-63 min after administration of curcumin (200 mg/kg, i.p.)

Many previous studies have reported the protective effect of curcumin on the central nervous system, which improves damaged neurons by anti-oxidant

and anti-inflammatory mechanisms as well as inhibits the expression of nuclear factor-kappaB [5]. In addition, curcumin, a plant based medicine, is well tolerated and has a low incidence rate of side effects. No previous studies have shown that high-dose curcumin causes toxic effects on humans or animals [8, 25]. The early studies [6] have indicated that curcumin protects rat hippocampal neurons from apoptosis. L-type Ca^{2+} channels are important ion channels existing in the postsynaptic membrane playing a major role in synaptic plasticity. It has been shown that curcumin decreases Ca^{2+} concentration in hippocampal synaptosomes [26]. Curcumin can also cross the blood-brain barrier [17].

Thus, the obtained data may serve as a basis for the further investigations of the neuroprotective action of curcumin against various neurodegenerative diseases.

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Կուրկումինի ազդեցությունը առնետների հիպոկամպի նեյրոնային ակտիվության վրա

Լ.Վ.Դարբինյան

Կուրկումինը *Curcuma longa*-ի ակտիվ բաղադրիչն է, և նրա նեյրոպրոտեկտիվ ազդեցությունը ցույց է տրվել առնետների և մկների մոդելներում:

Սույն հետազոտության նպատակն է հանդիսացել էլեկտրաֆիզիոլոգիական եղանակով գնահատել կուրկումինի ազդեցությունը էնտորինալ կեղևի բարձր հաճախականությամբ դրդման պայմաններում առնետների հիպոկամպի նեյրոնային ակտիվության վրա: Նյարդային ակտիվության փոփոխությունները գրանցվել են սուր փորձերի ժամանակ կուրկումինի ներարկումից անմիջապես հետո (200 մգ/կգ, ներորովայնային):

Կուրկումինի ազդեցությունները փոխկապակցված են բջջային գլյուտամատային մակարդակներում տեղի ունեցող փոփոխությունների հետ: Կուրկումինի չափաբաժնի ավելացմանը զուգընթաց հայտնաբերվել են նաև արգելակող ազդեցություններ: Բացի այդ, մենք ևս ենթադրում ենք, որ կուրկումինի էլեկտրաֆիզիոլոգիական ազդեցությունները կապված են ուղեղից ստացված ներքին նեյրոսնուցող ֆակտորների մակարդակների կարգավորման և առնետների մոտ հիպոկամպում ֆոսֆորիլացված ցիկլիկկաղենոզինմոնոֆոսֆատ-կապված պրոտեինի նվազեցված հարաբերակցության հետ:

Այսպիսով, առնետների մոտ կուրկումինի կարգավորիչ ազդեցությունը պայմանավորված է նեյրոփոխադրիչ մակարդակում տեղի ունեցող փոփոխություններով:

Воздействие куркумина на активность гиппокампальных нейронов у крыс

Л. В. Дарбинян

Куркумин является активным компонентом куркумы, и его нейропротекторное действие было показано в моделях крыс и мышей. В данном исследовании мы оценивали способность куркумина модулировать электрическую активность гиппокампальных нейронов.

Изменения нейрональной активности были зарегистрированы при введении куркумина (200 мг / кг, внутрибрюшинно). Эффекты куркумина коррелируются с изменениями клеточного уровня глутамата. Ингибирующие эффекты также были увеличены в отношении дозы куркумина. Мы полагаем, что электрофизиологические эффекты куркумина обусловлены снижением уровня нейротропного фактора мозга – протеина, который способствует выживанию существующих нейронов, появлению и дифференцированию новых соотношений концентрации цАМФ-элементсвязывающего белка в гиппокампе крыс.

Таким образом, модулирующие эффекты куркумина у нормальных крыс обусловлены колебаниями уровня нейротрансмиттеров.

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