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Effects of phenytoin on morphology and structure of hippocampal CA3 pyramidal neurons of rats in chronic stress

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KEY WORDS phenytoin; hippocampus; neurons; stress

ABSTRACT

AIM: To investigate the effects of phenytoin (DPH) on morphological and structural changes of pyramidal neurons in hippocampal CA3 of rats induced by chronic stress. **METHODS:** Using Nissl staining, Golgi staining, and electron microscope, the morphology and structure of pyramidal neurons in hippocampal CA3 of rats were observed. **RESULTS:** Chronic stress resulted in loss of hippocampal CA3 pyramidal neuron from 39 ± 4 to 35 ± 4 , shortening of total length of apical dendrite (from $196 \mu\text{m} \pm 35 \mu\text{m}$ to $156 \mu\text{m} \pm 33 \mu\text{m}$, $P < 0.05$), and ultrastructural degenerations of neurons. DPH markedly inhibited the decreases in number of hippocampal CA3 pyramidal neuron (38.4 ± 2.2) and total length of apical dendrite ($198 \mu\text{m} \pm 36 \mu\text{m}$, $P < 0.05$), meanwhile, improved neuron ultrastructural degenerations caused by chronic stress. **CONCLUSION:** Chronic stress does damage to hippocampal CA3 pyramidal neurons and DPH protects hippocampus from damage induced by chronic stress.

INTRODUCTION

Stress, especially chronic stress, is a precipitative cause of many mental disorders such as recurrent depression and posttraumatic stress disorder (PTSD)^[1,2], and many physical disorders such as cardiovascular, endocrinic, and gastrointestinal disorders. Hippocampus plays a vital role not only in learning, memory, endocrinic, and visceral functions, but also in regulating negative feedback of hypothalamic-pituitary-adrenal (HPA) axis, which is the endocrinic component of stress response^[1,3,4]. The interplay between the two functions of the hippocampus, its role in learning and memory and in modulation of stress response, becomes a subject of great interest in recent years. Hippocam-

pus is an especially plastic and vulnerable brain structure that could be damaged by many injurious factors^[5,6]. It is helpful to investigate the changes of neural morphology and neurochemistry of hippocampus induced by chronic stress for revealing the pathogenesis of stress-related mental disorders such as recurrent depression and PTSD. It was reported that chronic stress induced atrophy of apical dendrites of hippocampal CA3 neurons and alteration of synaptic terminal ultrastructure, and DPH, an antiepileptic drug, inhibited stress-induced atrophy of apical dendrites of CA3 pyramidal neurons^[7-9]. But little is known about the effects of stress on ultrastructure of neuronal perikaryon and the effects of DPH on comprehensive structure of neurons in hippocampal CA3 in chronic stress. The purpose of this study is to investigate the effects of DPH on morphological and structural changes of hippocampal CA3 pyramidal neurons of rats induced by chronic stress.

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MATERIALS AND METHODS

Drugs and reagents DPH was obtained from Yancheng Pharmaceutical Co Ltd (China). It was dissolved in distilled water and autoclaved before use. Toluidine Blue O was purchased from AMRESCO Biotechnology Co (USA). Other reagents were of analytic grade.

Animals treatment Thirty-eight adult male Spargue-Dawley rats (weighing 180-240 g, Grade II, Certificate No 20000008) were purchased from Experimental Animal Center, the Sun Yat-Sen University of Medical Sciences. The rats were randomly divided into three groups: control, stress, and stress+DPH, 12-13 rats in each group. Before experiment all rats were housed quietly for one week for habituation, and kept on a light/dark cycle of 12/12 h, free access to food and water.

The rats in stress group and stress+DPH group were subjected to forced-swimming for 15 min (8:00-10:00 am) daily for four weeks. A glass tank (40 cm×80 cm) containing 20-cm depth water maintained at 25 °C±1 °C was used. Rats in stress+DPH group received ip injections of DPH (40 mg/kg, 10 mL/kg)^[10] before forced-swimming. The other groups received ip injections of distilled water (10 mL/kg).

Nissl staining procedure The rats were anesthetized with ethyl ether and decapitated. The left-brains were embedded in paraffin. The 6-μm thick sagittal sections were cut within 1-4 mm nearer position of the sutura sagittalis. Four sections at equal interval were selected for Nissl staining. According to the regular method, the Nissl staining was carried out for counting the number of hippocampal CA3 pyramidal neurons.

Two continuous fields in hippocampal CA3 sub-region were selected for each section and the neurons were counted using the optic microscope at 400× magnification with putting the chord of arc on the diameter of ocular. The mean of two fields was taken as the neuron number of this section and the mean of four sections was taken as the neuron number of this specimen.

Golgi staining procedure The right-brains were cut into 5-mm thick blocks, namely 1-6 mm nearer position of the sutura sagittalis. The blocks were immersed in 3 % potassium dichromate solution at 37 °C for 3 d, and then transferred into 1.5 % silver nitrate solution and stored in the dark at 37 °C for 3 d. The two solutions were changed every day. After embedded in

paraffin, the blocks were cut into 25-μm thick sagittal sections. The sections were deparaffined in xylene and coverslipped directly with neutral balsam.

Computerized image technique Referring to Magarinos' s methods^[7] and using computerized Image Analysis System (Quantimet-520, Cambridge Instruments Company, UK) with Jandel Sigma Scan Pro software (American SPSS Inc), the total length of apical dendrite and the diameter of primary dendrite were measured. The total length of apical dendrite is the farthest distance from the beginning of primary dendrite to the end of last branch. The diameter of primary dendrite is the width of midpoint between its beginning and its initial branch point.

Electron microscopy Hippocampal formations of rats ($n=2$ per group) were sliced perpendicular to the septotemporal axis, in 2 % precold glutaraldehyde, at approximately 1-mm thick. After 30 min, 1-mm³ blocks in hippocampal CA3 were cut again. The samples for transmission electron microscopy were prepared according to standard procedures.

Statistical analysis The number of neuron, the total length of apical dendrite, and the diameter of primary dendrite, were analyzed statistically by one-way analysis of variance (ANOVA) with SPSS 10.0. Results were presented as mean±SD.

RESULTS

Assay of number of hippocampal CA3 pyramidal neurons The arrangement of hippocampal CA3 pyramidal neurons of control group was trim and dense and the Nissl substance in cytoplasm was clearly discernible (Fig 1A). The arrangement of pyramidal neurons of stress group was sparse and the Nissl substance was decreasing or dissolving (Fig 1B). The arrangement of hippocampal CA3 pyramidal neurons of stress +DPH group was better than that of stress group (Fig 1C). The number of hippocampal CA3 pyramidal neurons of stress group (35±4, $n=11$) was significantly less than that of control group (39±4, $n=11$) and stress+DPH group (38.4±2.2, $n=10$) ($P<0.05$). Difference was not significantly detected between control group and stress+DPH group ($P>0.05$).

Assay of total length of apical dendrite and diameter of primary dendrite of hippocampal CA3 pyramidal neurons The total length of apical dendrite of stress group was significantly shorter than that of control group and stress+DPH group ($P<0.05$). Dif-

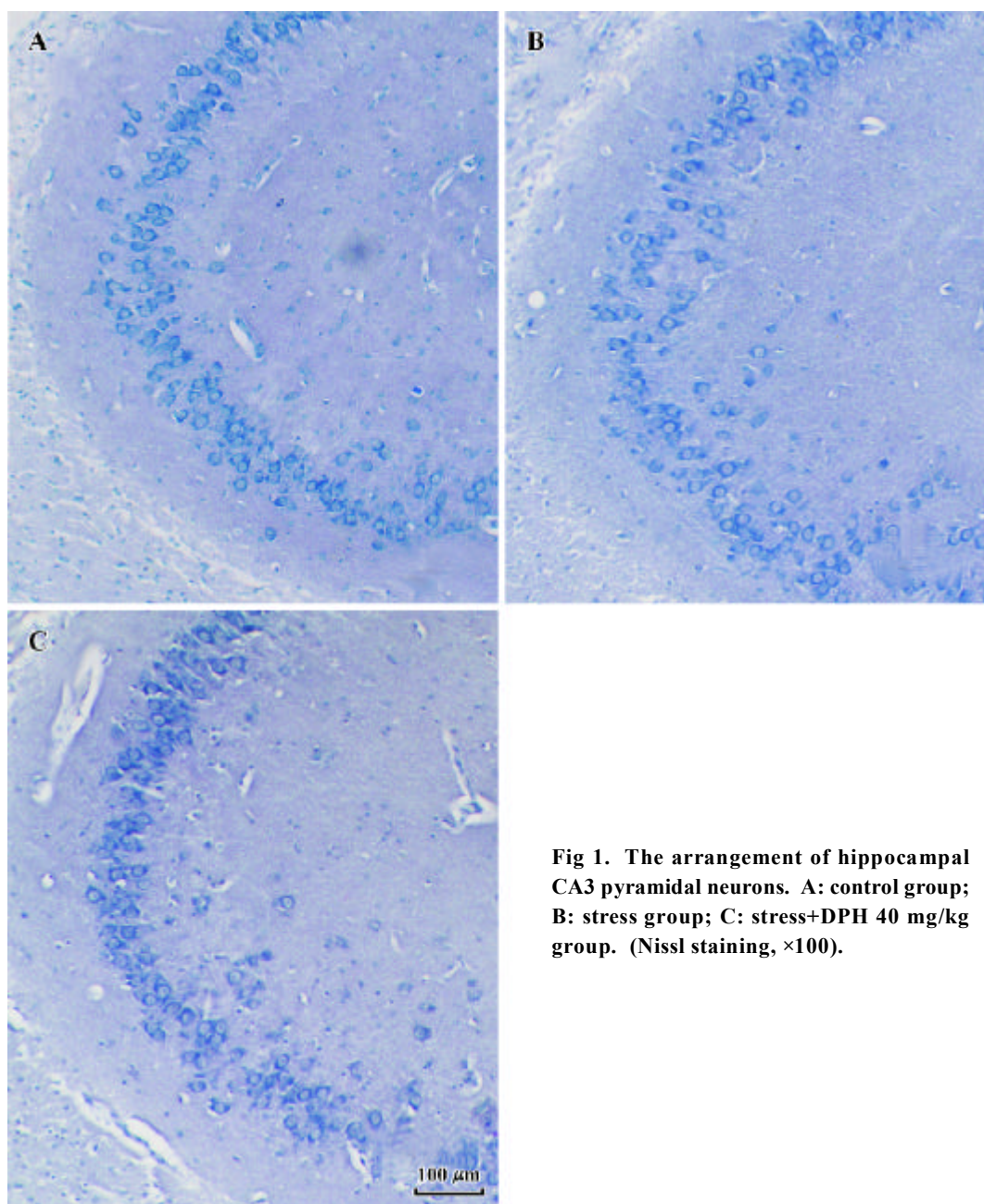


Fig 1. The arrangement of hippocampal CA3 pyramidal neurons. A: control group; B: stress group; C: stress+DPH 40 mg/kg group. (Nissl staining, $\times 100$).

ference was not significant between control group and stress+DPH group. The diameters of primary dendrite had no differences among three groups ($P > 0.05$, Tab 1).

The ultrastructure of pyramidal neurons The ultrastructure of pyramidal neurons in control group is normal (Fig 2A). The ultrastructural changes, including the condensation of the cytoplasm, the reduction of the cell volume, the dissolution of plasmalemma, the wavy shrinkage of the nuclear membrane, the reduction of the rough endoplasmic reticulum (namely Nissl substance), and the vacuolization of mitochondria with destruction of crista, were observed in hippocampal

Tab 1. Comparisons of total length of apical dendrite and diameter of primary dendrite of hippocampal CA3 pyramidal neurons among three groups. $n=10-11$. Mean \pm SD. ^b $P < 0.05$ vs control. ^c $P < 0.05$ vs stress.

Group	Total length of apical dendrite/ μm	Diameter of primary dendrite/ μm
Control	196 \pm 35	5.3 \pm 1.0
Stress	156 \pm 33 ^b	6.2 \pm 1.0
Stress + DPH 40 mg/kg	198 \pm 36 ^c	5.8 \pm 1.0

pyramidal neurons of stress group (Fig 2B, 2C). In stress+DPH group, no distinct ultrastructural damage was found in pyramidal neurons: the nuclear membrane was smooth, the mitochondria had no notable change, and the rough endoplasmic reticulum was abundant (Fig 2D).

DISCUSSION

Hippocampus is a target of stress hormones, which makes it liable to damage during chronic exposure to many neurological challenges such as stroke, hypoxia, head trauma, epilepsy, and aging^[5]. This study con-

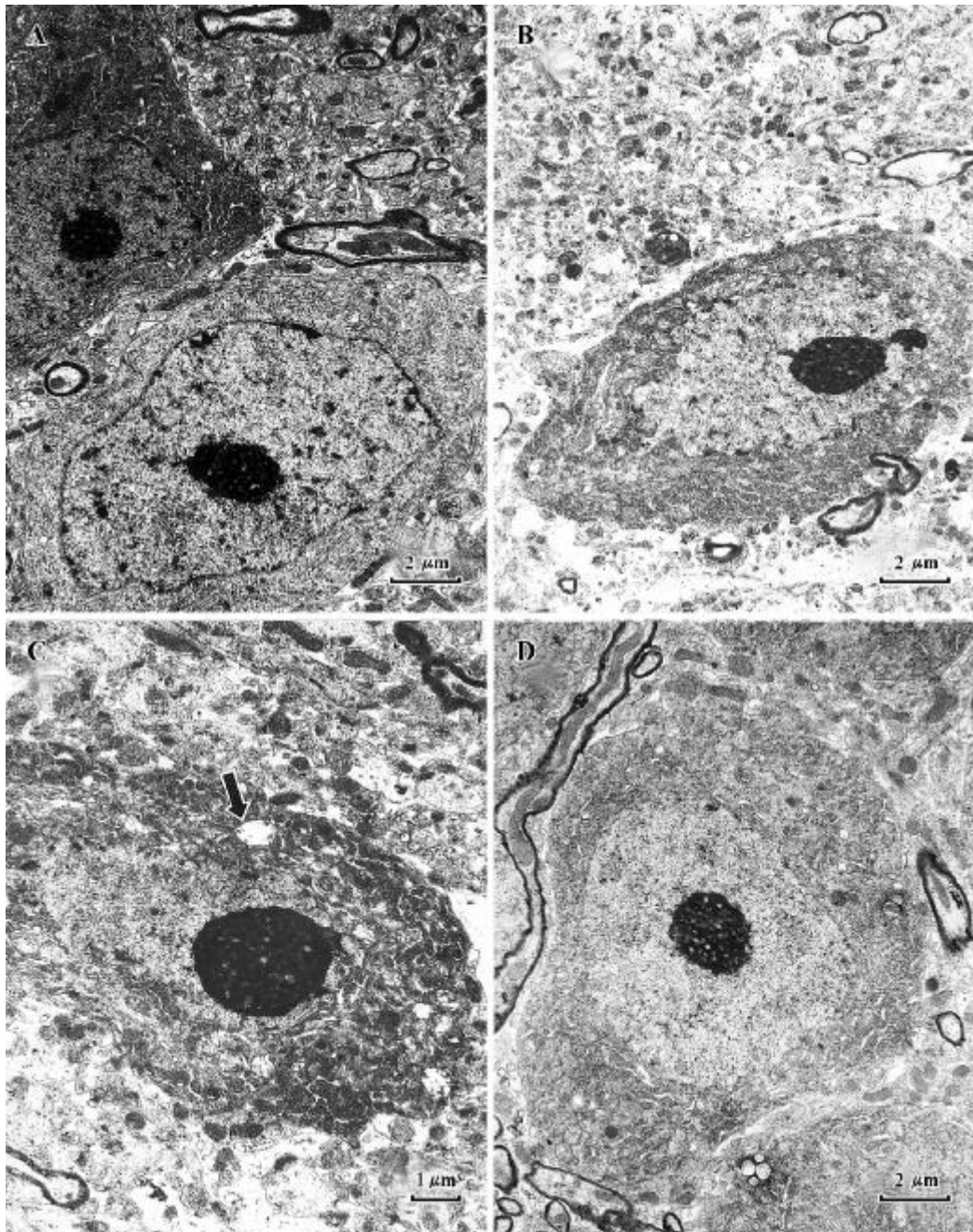


Fig 2. The ultrastructure of hippocampal CA3 pyramidal neurons. A: control group (×5000); B and C: stress group (×5000 and ×7000, respectively); D: stress+DPH 40 mg/kg group (×5000). Electron microscopy. Arrow shows vacuolar mitochondrion.

firmed results from other laboratories that chronic stress induced loss of hippocampal neurons and atrophy of apical dendrites in length^[7,11-13]. At the same time, we detected that the diameter of primary dendrite, as one of the active parameters of pyramidal neurons, was not affected by chronic stress. Magarinos *et al* reported that chronic stress resulted in rearrangement of synaptic terminal vesicles in electron microscope^[8]. Our study further showed that chronic stress induced ultrastructural changes in neuronal perikaryon itself in hippocampal CA3, including the condensation of the cytoplasm, the wavy shrinkage of the nuclear membrane, the vacuolar degeneration of the mitochondria, and the reduction of the rough endoplasmic reticulum, which possibly would lead to neuronal death and loss finally.

DPH, an anti-epileptic drug, is well known to suppress the release of glutamate (Glu) and block activation of T-type calcium channels. According to previous data, DPH inhibited stress- and glucocorticoid-induced atrophy of CA3 pyramidal neurons in rats and in tree shrews^[9,13]. Besides supporting these results, this study also displayed that administration of DPH before stress inhibited the loss of hippocampal neurons and the ultrastructural degenerations of neuronal perikaryon. It indicated that DPH could almost completely protect hippocampus from damage induced by chronic stress. The protective effect of DPH on hippocampus in chronic stress in turn demonstrated that Glu release could be activated in response to stress. Moreover, this inference is further supported by the facts that Glu level in the hippocampus increased in stress and that Glu receptor blockades prevented stress-induced atrophy of apical dendrites of CA3 neurons in the hippocampus^[14,15]. Thus, combining the present study with previous data reported by others, we think accumulation of Glu and influx of Ca²⁺ might play a critical role in triggering the morphological and structural alteration of CA3 neurons in the hippocampus during exposure to chronic stress.

Preclinical and clinical studies of recurrent depression and PTSD found the atrophy and death of neurons and the reductions of volume in hippocampus, which suggests that the occurrence of hippocampal damage may be correlative with later mental disorders^[2,16]. Thereby, the agents that inhibit hippocampus from damage, such as DPH, might be taken as a treatment for the mental disorders associated with chronic stress.

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