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Original Research

Fluoxetine inhibits dendrite atrophy of hippocampal neurons by decreasing nitric oxide synthase expression in rat depression model[1]

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KEY WORDS fluoxetine; antidepressive agents; hippocampus; neurons; nitric-oxide synthase

ABSTRACT

AIM: To study the effect of fluoxetine on dendrite atrophy of hippocampal neurons in rat depression model. **METHODS**: CMS (chronic mild stress), mimicking human depression, was used as the animal depression model. The neurons shape and numbers of nitric oxide synthase positive cells in the hippocampal subfields were measured by Nissl staining and histochemical staining of NADPH (nicotinamide adenine dinucleotide phosphate)-diaphorase respectively. **RESULTS**: CMS deforms neurons in the hippocampal formation, and fluoxetine can renormalize the deformed neurons by inhibiting the nitric oxide synthase catalyzing the over-production of NO, which lead subsequently to the morphological abnormality in the circumscribed area of brain. **CONCLUSION**: Fluoxetine, an antidepressant, renormalizes dendrite atrophy of hippocampal neurons by inhibiting nitric oxide synthase overexpression in rat chronic mild stress model.

INTRODUCTION

Depression, a recurrent mental disorder of surprisingly great prevalence, remains inadequately managed by the present drug therapy[1-3]. This disease is thought to be associated with the metabolism of monoamine neurotransmitters such as 5-HT (serotonin), NE (norepinephrine), and DA (dopamine), thus setting up the framework of the update understanding of depression[4]. Presently, most conventional antidepressants, though structurally diversified, are believed to be "monoamine based," namely, to improve the synaptic availability of 5-HT, NE, and/or DA. However, the evidences of the monoamine function are largely indirect[5,6], and other neurotransmitters as well as hormones are considered to be involved in pathophysiology of depression, too[7,8]. Strangely, tianeptine, an enhancer of 5-HT reuptake in brain, has been dramatically developed as a clinically efficacious antidepressant[9]. Accordingly, the monoamine hypothesis of depression has to pave the way for more comprehensive view(s) of the brain disorder, underlying that the incidence of depression is also dependent on some other physiological dysfunctions[10].

Fluoxetine (commercially called 'prozac') is the most frequently prescribed antidepressant that was shown to work as a selective 5-HT reuptake inhibitor. Besides, the drug is also capable of inhibiting the NE reuptake and 5-HT2C receptor[4]. Pharmacokinetically, the drug elevates the concentration of 5-HT at the synapses within a few hours after administration. However, patients have to keep taking the medicine for 2-3 weeks before they can experience its antidepressant effect. This indicates that the symptom of depression does not disappear rapidly upon the elevation of 5-HT levels. Thus, the update knowledge regarding the antidepressant mechanism of fluoxetine seems to be quite limited. We therefore investigated the effects of fluoxetine on hippocampal pyramidal neurons *in vivo* in order to obtain a better understanding of its antidepressant action that would provide new clues valuable for the research and development of new antidepressant(s).

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MATERIALS AND METHODS

Subjects Thirty male Sprague-Dawley rats (Grade Ⅱ, Certification № 97001, Nanjing Medical University) weighing 250 g ± 20 g were singly housed in perspex cages (35 cm × 24 cm × 8 cm) and maintained on a 12 h light/dark cycle with food and water freely available.

Chronic mild stress (CMS) CMS is a depression mimicking animal model described earlier by Willner *et al*[11] where the depressiveness of tested rats is usually monitored by a decrease in the consumption of the palatable dilute (1 %) sucrose solution. Technically, all rats were first trained to consume the sweet liquid by making them freely access to 1 % sucrose solution 1 h daily in 10 successive days, each after a 14-h food and water deprivation. The amount of sucrose solution drunk by each rat was recorded during that 1-h accession. On the basis of their sucrose intake in the final test, the animals were divided into two groups amounting to 20 and 10 rats, respectively. That of 20 animals was subjected to CMS for 6 weeks. The stress regime of each week included actually two random combinations of the following separately delivered stresses (14 h each): food and water deprivation (one of which was immediately prior to the sucrose intake test), soiled cage, 45° cage tilt, paired housing, low intensity stroboscopic illumination, and intermittent overnight lightening. Normal control group, housed in another room, was exclusively deprived of food and water for 14 h prior to the sucrose intake test. After 3-week exposure to the stress, the animals were divided into two equal subgroups, one treated with flouxetine (1.8 mg·kg^{-1}·d^{-1}, ig, set according to the dosage for patients) and the other with vehicle (1.5 mg·kg^{-1}·d^{-1}, ig).

Histological and histochemical procedures Twenty-four hours after the final injection, all animals anaesthetized with 1 % pentobarbital sodium were perfused with 0.9 % NaCl and subsequently with 500 mL of fixative containing 4 % paraformaldegyde in PBS 0.1 mol/L (phosphate buffer solution, pH 7.4) via aorta for 40 – 50 min. All brains were removed and stored in the same fixative for 2 h, and then transferred into 30 % sucrose followed by standing overnight. Coronal sections were cut at 40 μm thickness in a cryostat, and every eight sections two were collected. One section was prepared for Nissl staining and the other for NADPH (nicotinamide adenine dinucleotide phosphate)-diaphorase modified from Kato's method[12]. The Nissl staining was carried out according to the regular cresylecht violet staining method with hippocampal pyramidal neurons observed under an Olympus microscope. As to histochemical staining of NADPH-diaphorase, sections were incubated for 90 min at 37 ℃ in freshly prepared PBS 0.1 mol/L containing β-NADPH 1 g/L, nitroblue tetrazolium 0.25 g/L and 0.4 % Triton X-100. After being rinsed sequentially in PBS 0.1 mol/L, distilled water, and acetic acid 0.2 mol/L (2 min each), the sections were mounted on slides pre-coated with 0.5 % gelatin solution followed by drying. Finally the sections were dehydrated through a graded series of alcohol and coverslipped for microscopic observation. Quantification of NADPH-diaphorase positive neurons in hippocampal subfields CA1, CA2 – 3, and dentate gyrus (DG) was carried out as reported (Fig 1).

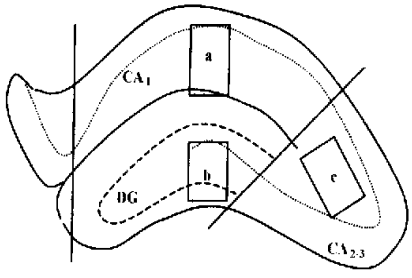

Fig 1. The NADPH-diaphorase positive neurons counting area of hippocampal subfields (a, b and c represent the observed area).

Statistics Results of sucrose intake were analyzed by analysis of variance, and numbers of NADPH-diaphorase cells by *t*-test.

RESULTS

The effect of fluoxetine on the sucrose intake A 3-week application of the stress to rats led to a significant reduction in their sucrose consumption (Fig 2). The decrement remained for stressed animals during the follow-up treatment with vehicle. However, administration of fluoxetine to the stressed rats caused a gradual increment in the sucrose intake, finally up to the amount consumed by the control group without pre-stressing (Fig 2). The results ascertained the development of anhedonia of the animals due to the three-week exposure to the stress regime, and this behavioral deficit could be reversed by fluoxetine.

Changes of hippocampal pyramidal neurons As shown in Fig 3, the hippocampal pyramidal neurons

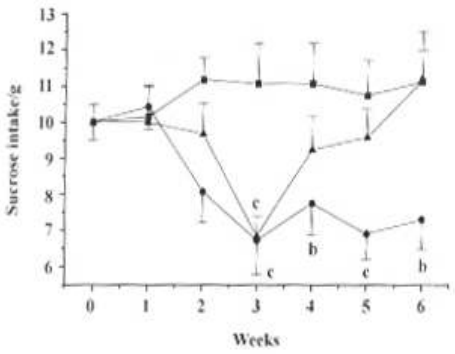

Fig 2. The decrement in 1 % sucrose solution consumption within a 1-h test in the CMS model and its increment by the treatment with fluoxetine. CMS applied rats treated with fluoxetine (▲) and vehicle (●) respectively, and normal control rats (■). Results were analyzed by analysis of variance. $n = 10$. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* the normal control group.

of stressed animals treated with vehicle are markedly different from control and fluoxetine administered groups. In this group of animals, dendrites of neurons in CA1, CA2, and CA3 regions atrophied so severely that the cells deformed apparently. However, there is no discernible difference between the neurons in that part of control and fluoxetine treated animals, indicating that the atrophy thereon disappeared upon the treatment with fluoxetine.

Changes of the NADPH-diaphorase positive neurons in hippocampus The NADPH-diaphorase positive neurons in hippocampus were shown in Fig 4. In CA1 and CA2-3 regions of hippocampus, the number of NADPH-diaphorase positive neurons discerned in chronic mild stressed animals treated with fluoxetine was close to that of the normal control group whereas a striking increment in that of stressed rats treated with vehicle was observed (Fig 5). There is no substantial difference among all three groups in the number of the neurons in dentate gyrus. The number of NADPH-diaphorase positive neurons represented the expression of NOS (nitric oxide synthase) was described as before[12,13].

DISCUSSION

CMS model is a valid model of anhedonia that is a core symptom of depression characterized by the decreased appeal and ability to experience pleasure[14,15]. Chronic application of some mild stresses to rats causes a

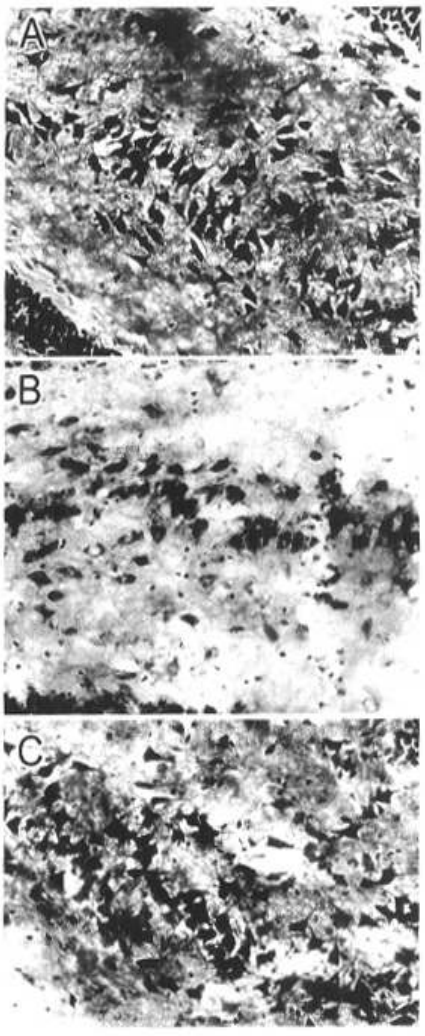

Fig 3. The deformation of pyramidal neurons in hippocampus following CMS and its recovery by the treatment with fluoxetine. The cells were shown by Nissl staining method. A) The normal control animal. B) The chronic mild stressed animal treated with vehicle. C) The chronic mild stressed animal treated with fluoxetine. × 350.

significant decrease in responsiveness to rewards, which is usually monitored by consumption of the palatable sucrose solution.

In view of the facts that some stresses such as restraint, electric shock, and cold water swimming can lead to injuries to the neuron in limbic forebrain of animals[16,9], and that a stressful event can also be a biological cause of human depression[17,18], we investigated the presumable neuronal deformation resulted from

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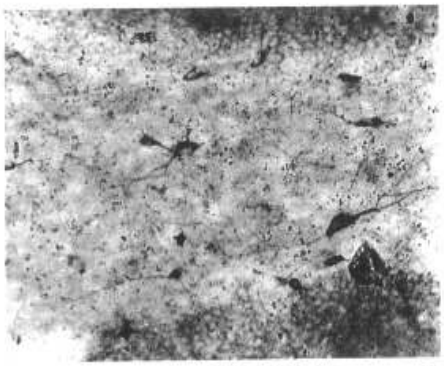

Fig 4. Photomicrograph of NADPH-diaphorase positive neurons in hippocampus of rats. There are two different types of labeled cells. Type Ⅰ consisted of big-sized Golgi-like cells which is stained intensely, and Type Ⅱ of small and lightly stained cells. ×260.

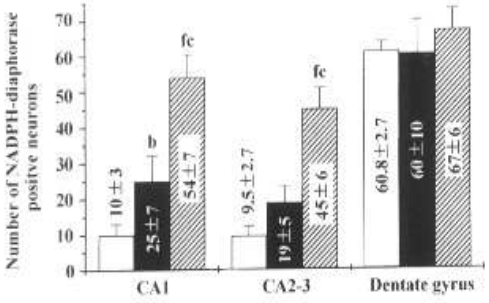

Fig 5. Changes of numbers of NADPH-diaphorase positive neurons in hippocampal subfields (CA1, CA2-3, and dentate gyrus) after CMS and fluoxetine treatment. Normal control (□), CMS-applied animals treated with fluoxetine (■) and vehicle (hatched bars). $n = 10$ rats. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* control. $^fP < 0.01$ *vs* CMS group treated with fluoxetine.

CMS and the neuroprotectivity of fluoxetine. Although it is generally accepted that increasing the concentration of monoamine in the brain is assumed to be an effective way to alleviate or cure depression with most current antidepressants being monoamine regulators, the incidence of depression has already been shown to be more complex than the insufficiency of monoamine neurotransmitter(s)[19-21]. In the present investigation, the deformed hippocampal neurons of animals with anhedonia induced by CMS was discerned. Both dendrite atrophy and cell deformation were detected suggesting effects of CMS on the normal neuron structure in the circumscribed area. As the normal function of brain depends on the integrity of neurons and their circuit, we thus hypothesize that the antidepressant may exert its effects on these brain areas at least in part by rebuilding the normal structure. Concerning fluoxetine, the present study demonstrated that it renormalized atrophied hippocampal neurons in the rat CMS model. This finding explained the delayed efficacy of most antidepressants since it takes time to "reconstruct" the injured neuronal connections. Furthermore, the newly reconstructed neuron connections could be incomplete and/or reversible, raising therefore difficulties in current depression management. The result of our experiment suggested that the deformation of neurons in some brain areas is one of the indicators of depression development.

The subsequent interesting question is how fluoxetine inhibited the hippocampal neuron atrophy in the CMS model. Previous investigations have revealed that NO (nitric oxide) has been implicated both as a neuromodulator and a mediator of neurotoxicity in the central nerve system[22,23] and that NOS (nitric oxide synthase), a key enzyme catalyzing NO production, has been shown to distribute widely in brains[24]. As a reinforcement of the observation, chronically applied stresses can lead to a discernible morphological deformation of the rat hippocampal neurons, which could be inhibited by NMDA (*N*-methyl-*D*-aspartate) receptor antagonists[25]. Some NMDA receptor antagonists are preclinically and clinically disclosed to be as efficacious as tricyclic antidepressants, and are hopeful to become a new antidepressant[26]. Physiologically, the NMDA receptor complex increases influx of Ca^{2+}, which interacts with calmodulin to activate NOS, indicating that the enzyme is presumably involved in the development of depression. The reaction of NO with superoxide can yield a variety of highly toxic molecules like $ONOO^-$ that may mediate the neuronal injury or damage[27]. As a confirmation of this assumption, some NOS inhibitors such as *L*-NNA [*N*(G)-nitro-*L*-arginine], its dextrorotatory enatiomer *D*-NNA, *L*-NAME [*N*(G)-nitro-*L*-arginine methyl ester], and *L*-NMMA [*N*(G)-monomethyl-*L*-arginine] have been reported to possess antidepressant-like properties with the efficacy comparable to that of imipramine in the mouse forced swimming test[26]. The follow-up chronic treatment test showed that these inhibitors could down-regulate cortical beta-adrenoceptors[28]. Technically, the magnitude of the NOS expression is usually indicated by the number of NADPH-diaphorase positive neurons[12,13]. Our experiment in this respect showed that fluoxetine reduced the number of NADPH-diaphorase positive neurons in CA1

and CA2 – 3 of hippocampus demonstrating that stresses such as CMS atrophy hippocampal neurons, and that fluoxetine can renormalize the deformation by inhibiting most probably the NOS to protect thereby the circumscribed area of brains from the NO in toxication.

The present study renews the understanding of the antidepressant mechanism of fluoxetine and pathophysiology of depression. Furthermore, this research also offers a new clue for the research and development of new antidepressant with more rapid onsets of action, higher response rates, and better long-term efficacy.

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氟西汀通过降低一氧化氮合酶的表达而抑制抑郁症模型大鼠海马神经元树突萎缩[1]

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关键词 氟西汀；抗抑郁药；海马；神经元；一氧化氮合酶

目的：研究氟西汀对抑郁症大鼠模型的海马神经元树突萎缩的作用. **方法**：用慢性温和性应激模型作为抑郁症模型，采用尼氏染色观察海马各亚区神经元形态，用 NADPH-d 组化染色方法测定了海马中一氧化氮合酶阳性神经元的数量. **结果**：慢性温和性应激对海马神经元有一定的损伤，主要表现在神经元树突的萎缩，而氟西汀可使这些受损神经元恢复正常，这种作用与氟西汀抑制海马区的一氧化氮合酶阳性神经元的数量减少相关. **结论**：氟西汀可通过抑制海马区一氧化氮合酶的过度表达而阻止或扭转抑郁症模型大鼠海马神经元树突的萎缩.

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