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SUCROSE PREFERENCE IS RESTORED BY ELECTRO-ACUPUNCTURE COMBINED WITH CHLORIMIPRAMINE IN THE DEPRESSION-MODEL RATS

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ABSTRACT:

The present study was to investigate the effect of electroacupuncture (EA) combined with chlorimipramine on the sucrose preference of depressive rats induced by chronic mild stress (CMS). Rats were exposed chronically (1^{st} –4^{th} week) to a variety of mild unpredictable stresses. The tricyclic antidepressant chlorimipramine and EA were administrated on these depressive rats for 6 weeks (5^{th} –11^{th} week). EA was applied at points of "Bai-Hui" (Du 20) and "An-Mian"(EX 17) (right side), by EA apparatus (Model 6805-2, Shanghai). Dense (60Hz/5sec)-sparse (4Hz/2.5sec) frequency of the wave was selected and the current intensity (≤1mA) was adjusted to provoke slight twitches of the rat's ear. The preference for 1% sucrose solution and the immobility time in the forced swimming test were measured as the symptoms of anhedonia and depressed mood, which were central features of major depression. The preference for 1% sucrose solution was reduced by CMS, but could be restored to normal level after 6 weeks treatment with chlorimipramine at 5mg/kg or EA combined with

chlorimipramine at 2.5mg/kg. In the forced swimming test, the immobility time of depressive rats was decreased in both groups. However, the preference for sucrose and the immobility time in the depressive rats were not significantly changed by the treatment with only EA or chlorimipramine at 2.5 mg/kg. The results suggested that EA could potentiate the antidepressant effect of chlorimipramine in low dose, and EA combined with antidepressant might be a better method in treating depression.

KEY WORDS: Depression, Antidepressant, Chlorimipramine, Electroacupuncture

INTRODUCTION

The prevalence of depression is consistently high worldwide, and is associated with considerable morbidity and mortality. In the United States, the estimated lifetime prevalence is 21.3% in women and 12.7% in men [1]. The goal of pharmacotherapy is the reduction and ultimate removal of all signs and symptoms of depression. There are now more than 2 dozen drugs that are available for treating depression, which belong to four different classes — tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), monoamine oxidase inhibitors (MAOIs) and miscellaneous antidepressants. However, in China, TCAs are chosen widely in clinical practice for the efficacy and the low cost. But, the current antidepressant drugs, including TCAs and the new, such as SSRIs, have common shortcomings from several clinical standpoints, such as 1) efficacy rate remains at 60-70%, 2) lack of immediate onset of efficacy and 3) side effects of drugs [2,3,4].

The use of complementary and alternative medicine (CAM) is widespread. Those with psychiatric disorders are more likely to use CAM than those with other diseases[5]. There are both benefits and limitations to CAM, such as: EA could alleviate the symptoms of depression with few side-effect but there is incomplete efficacy in many patients like other antidepressant [5,6,7]. In addition, certain psychiatrists advise clinicians may choose augmentation and combination strategies in treatment-resistant depressions [8], such as combination two classic antidepressants or combination two kinds of therapy. Therefore, we try to combine EA with antidepressants in treating depression.

In primary experiment, we found that EA could potentiate the effect of chloromipramine in forced swimming test [9]. Certain characteristics of the CMS model suggest it may be relatively realistic as a model of depression [10]. Therefore, this CMS model was used to investigate the effects of EA combined with chlorimipramine, one of TCAs, in depression.

MATERIALS & METHODS

1. Animal Sprague-Dawley rats (male n=60, 200-250g) from the Department of Experimental Animal, Medical Center of Fudan University were housed with free access to water and food, a 12h light/dark cycle, the room temperature maintained at about 25℃. Rats were divided into six groups: 1) normal group without any treatment (n=10); 2) chronic mild stress (CMS) group (n=10); 3) CMS + chlorimipramine (chl , i. p. 2.5mg/kg) group (n=10); 4) CMS + chlorimipramine (chl , i. p. 5mg/kg) group (n=10); 5) CMS + EA group (n=10); 6) CMS + EA + chlorimipramine (chl , i. p. 2.5mg/kg) group (n=10). All protocols were approved by the Committee on Research Animal Care of Fudan University, and followed by the principles and procedures outlined in the NIH Guide for the Care and Use of Laboratory Animals.

2. Procedure The standard CMS protocol, as described by Willner and his colleagues [11], consists of the sequential application of a variety of mild stresses: (1) food deprivation (Monday 3:00 pm—Tuesday 3:00 pm, Wednesday 5:00 pm—Friday 12:00am, Saturday 10:00am —Sunday 12:00 am) and (2) water deprivation (Monday 3:00pm—Wednesday 11:00 am, Wednesday 5:00 pm—Thursday 10:00 am, Saturday 10:00 am—Sunday 12:00 am), (3) continuous lighting (Tuesday 5:00 pm—Wednesday 10:00 am, Saturday 5:00 pm—Sunday 12:00 am), (4) cage tilt (30°)(Thursday 10:00 am—5:00pm, Sunday 5:00 pm—Monday 10:00 am), (5) paired housing(Thursday 5:00 pm—Friday 10:00 am), (6) soiled cage (100ml water spilled onto bedding)(Monday 5:00 pm—Tuesday 10:00 am), (7) exposure to reduced temperature (18°C)(Monday 3:00 pm—3:30 pm ,Thursday 10:00 am—10:30 am), (8) intermittent white noise (85 dB)(Tuesday 3:00 pm—6:00 pm, Friday 12:00 am—5:00 pm), (9) stroboscopic lighting (300 flashes/min) (Monday 10:00 am—12:00 am , Wednesday 10:00am —5:00 pm), (10) exposure to an empty water bottle following a period of water deprivation (Wednesday 10:00 am—11:00 am), (11) restricted access to food (scattering of a few 45 mg precision pellets in the animal's home cage)(Friday 10:00 am—12:00 am), (12) presence of a foreign object in the home cage(e.g., piece of wood or plastic)(Wednesday 5:00 pm—Thursday 10:00 am).

3. Fluid Consumption tests Seventy-two hours before the start of the experiment, animals were given a continuous 48h exposure to two bottles: one containing 1% solution of sucrose, the other tap water. The bottles were counterbalanced across the left or right sides of the feeding compartment. This procedure was adopted for two-bottle tests throughout the experiment. Prior to testing for fluid consumption, animals were deprived of food and water for 23h. Testing was carried out in the animals' home cage 6h into light cycle, from 2:00 pm to 3:00 pm every Tuesday. The volume of fluid consumption was measured repeatedly three times as the pre-administration control. Sucrose preference rate was calculated according to the formula:

%Preference =[(Sucrose Intake/Total Intake)×100%]

4. Forced Swimming Test Rats were placed individually in Plexiglas cylinder (height 40cm, dia 18cm), containing 17cm of water at 25°C and 15 min later they were removed to dry for 30min. After 1 week, the animals were replaced in the cylinders, and during 5-min testing, their behavior was quantified by measuring the time spent floating (immobility time). Floating was defined, as the amount of time the animal remained passive in the water. During this period, the animal rested, keeping its head on the surface of the water in a horizontal or vertical posture, with all four limbs motionless without sinking.

5. EA EA was applied at points of "Bai-Hui" (Du 20), (7 cun directly above the midpoint of the posterior hairline, in galea aponeurotica) and "An-Mian"(EX 17) (right side), [in the midpoint of the line between Fengchi (G 20) and Yifeng (SJ 17)], between muscle strenocleidomastoideus and muscle spleitus capitis) by EA apparatus (Model 6805-2, Shanghai). Dense (60Hz/5sec)-sparse (4Hz/2.5sec) frequency of the wave was selected and the current intensity (≤1mA) was adjusted to provoke slight twitches of the rat's ear. The EA administration was maintained for 40 minutes (Fig.1. 2).

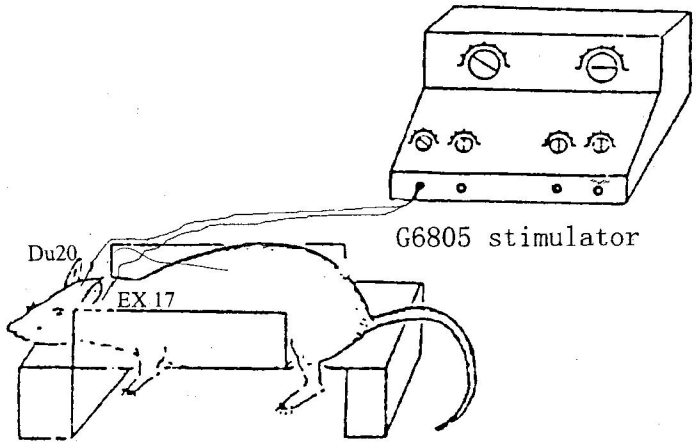

Fig.1 Schematic diagram showing EA procedures on rats

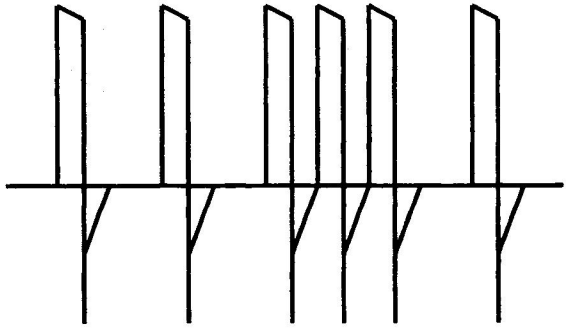

Fig. 2 the Stimulating wave-form of the EA

6. Treatment Drug and EA treatment started at the 5th week. Chlorimipramine was administered every day at 2.5mg/kg or 5mg/kg for 6 weeks. EA was applied Monday, Wednesday and Friday /each week for 6 weeks. The test of CMS was continued and sucrose consumption was measured at weekly intervals throughout 11 weeks.

7. Statistical analysis Data are presented as mean ± SE and analyzed by SPSS 10.0. Repeated measures analysis of variance (ANOVA) was used for overall effects, with S-N-K test for post-hoc analysis for differences between groups. $P < 0.05$ was considered statistically significant.

RESULTS

1. Fluid consumption

In the initial baseline test, the mean intakes of sucrose and water were 8.2±2.1ml and 1.9±1.1ml respectively, and the sucrose preference rate was 81±14.6%. After 4 weeks of stress exposure, sucrose consumption 3.2±1.4ml and sucrose preference rate 45±16.3% were decreased significantly in CMS group ($p<0.05$, vs control group) (Fig.3). But water consumption did not change obviously. When the treatments with EA, Chl 2.5mg/kg, Chl 5 mg/kg or Chl 2.5mg/kg+EA were performed, the sucrose intake began to increase at the end of the first week of treatment, however, the restore trend didn't keep up in EA and Chl 2.5mg/kg group. After 6 weeks of treatment, sucrose intake and sucrose preference rate were significantly restored in Chl 5mg/kg group and Chl 2.5mg/kg+EA group ($P<0.01$ vs CMS group) (Fig.4). The sucrose consumption was 7.8±1.4ml and 8.1 ±0.9ml respectively, and the value of sucrose preference rate was 84±9.3% and 78± 14% respectively.

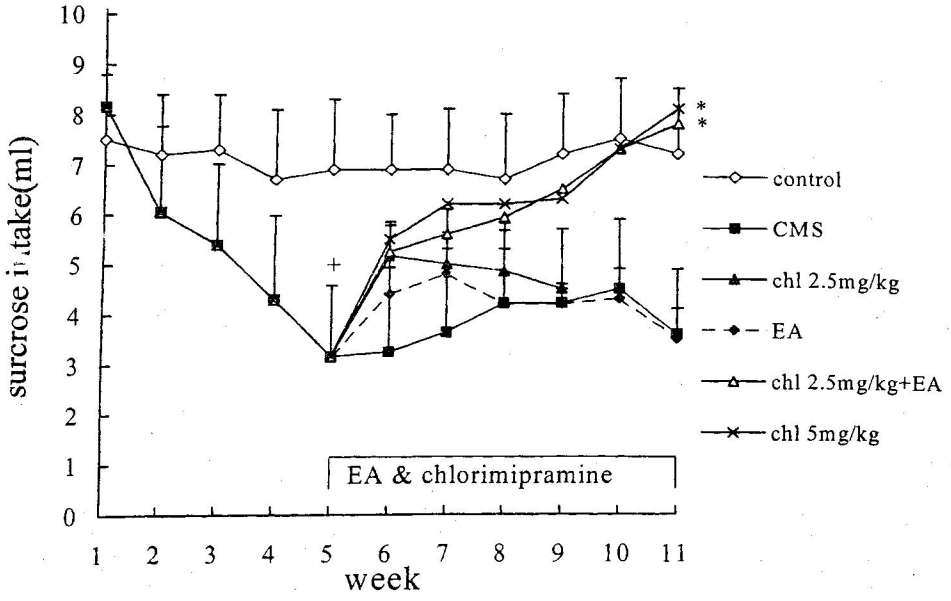

Fig.3 The effects of stress and treatment on sucrose (1.0%) consumption
+ $p<0.05$ vs control group * $p<0.05$ vs CMS group

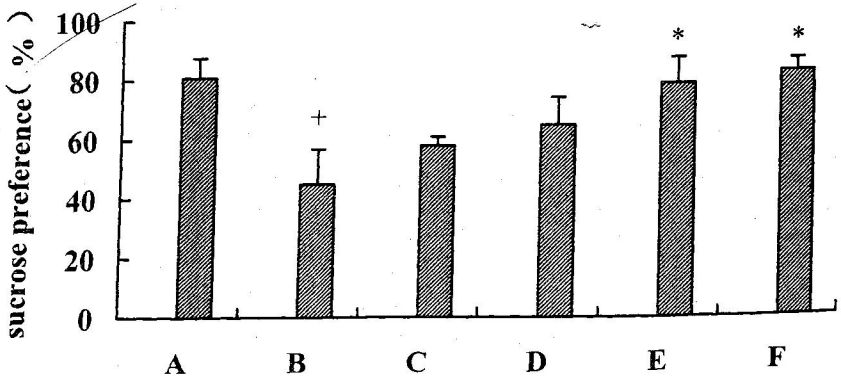

Fig. 4 The effects of stress and treatment on preference for sucrose (1.0%) over water
A: control group B: CMS group C: EA group
D: chl 2.5mg/kg E: chl 2.5mg/kg + EA F: chl 5mg/kg
+ $p<0.05$ vs control group * $p<0.05$ vs CMS group

2. Forced swimming test

After 11 weeks, the immobility time in water of the depressive rats and the treated depressive rats was measured. The immobility time of CMS group was 160±18.9s. The immobility time of Chl 5mg/kg group (90±21.6s) and Chl 2.5mg/kg+EA group (101.6±32.4s)were both decreased obviously ($p<0.05$ vs CMS group) (Fig.5). However, there is no significant change of immobility time between only CMS group and Chl 2.5mg/kg group.

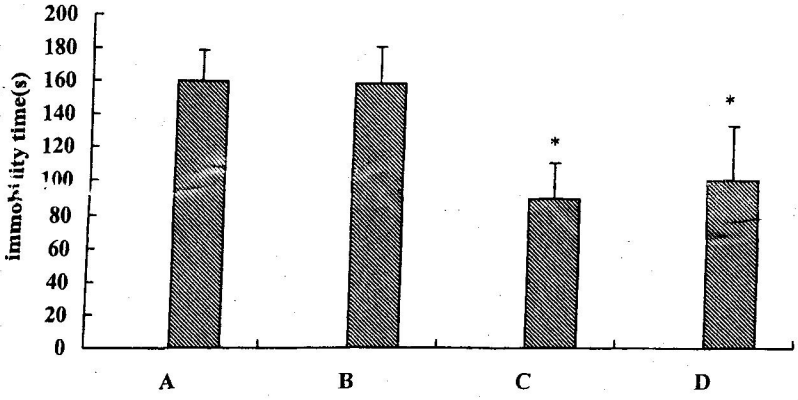

Fig.5 The effects of treatment on immobility time in the forced swimming test

A: CMS group B: chl 2.5mg/kg group
C: chl 2.5mg/kg + EA group D: chl 5mg/kg group
*$p<0.05$ vs CMS group

DISCUSSION

Certain characteristics of the CMS model indicated that it might be relatively valid as a model of depression. In particular, the stress regime is probably the most realistic experimental analogue available of the stresses of every day life, and may be analogous to the "life difficulties" implicated in the etiology of depression [10,12]. Furthermore, the use of a sweet reward and forced swimming test has some face validity in relation to the two core symptoms of major depression, i.e. anhedonia and depressed mood. The time courses of antidepressant such as chlorimipramine therapy accurately mimic the clinical situation. These parallels suggested that this CMS model could be used as screening tests in the context of antidepressant discovery and development programs, and as simulations within which to investigate aspects of depression.

Although options for pharmacological treatment for depression have grown seemingly exponentially over the past several decades, the current armamentarium of antidepressants continues to have limitations of both efficacy and tolerability. The problems include an unacceptable lack of efficacy, delayed onset of therapeutic effects, an inability to predict responses to one or another agent, drug-drug interactions, and difficulty with tolerability during both acute and chronic treatment. Most of patients cannot undergo the trail for the side effect of antidepressant, resulting in patients' lost of compliance. So, combination strategies are recommended, such as combination two therapies [13].

In some clinical experimental study, physicians investigated the effects of the acupuncture or EA on patients with depression. Their results indicated that acupuncture leads to a significant clinical improvement as well as certain antidepressants. Furthermore, a larger trial of acupuncture in the acute- and maintenance-phase treatment of depression was warranted before further recommendations can be made [6, 14]. Our experiment also indicated that EA can improve the symptoms of depression in rats of depression model but there was incomplete efficacy. The effects of EA are not more than the effects of chlorimipramine. In addition, the combination of EA and the chlorimipramine in low dose will potentiate the antidepressant effect of chlorimipramine. Germany Physicians also indicated additionally applied acupuncture improved the course of depression more than pharmacological treatment with mianserin alone [15].

Understanding the fundamental biology of major depression has proved to be a challenging scientific problem of enormous clinical relevance. Psychobiological research on depression has traditionally concentrated on the monoamine transmitter [16]. It has been long proposed that the depression in humans may result from deficient activity in the serotonergic and noradrenergic system, whereas mania is associated with a functional to excess of noradrenergic system. The action mechanisms of tricyclic antidepressant on clinical depression patients that augment monoamine function alleviate their symptoms. Chlorimipramine could inhibit the uptake of the serotonin and its metabolite and inhibit uptake of the norepinephrine which all could elevate the function of serotonin and norepinephrine. However, the increasing function of monoamine also induces many adverse effects such as side effect on autonomic nervous system and central nervous system. EA could change the release of monoamine transmitter at synaptic junctions, which could increase the release of serotonin but decrease the release of norepinephrine [17]. It is perhaps the reason that EA could potentiate the effect of chlorimipramine. Thus, the doses of chlorimipramine were reduced obviously, and subsequently the side effect of chlorimipramine was decreased. In addition, it is most important that EA has the regulatory role on body functions. This regulatory role makes it possible that EA could potentiate the clinical effect on the depression, accordingly, diminish the side effect of chlorimipramine.

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