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Inhibitory effect of jujuboside A on penicillin sodium induced hyperactivity in rat hippocampal CA1 area *in vitro*[1]

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KEY WORDS jujuboside A; hippocampus; neural inhibition; GABA

ABSTRACT

AIM: To study the effect of jujuboside A (JuA), one constituent of Chinese herbal medicine *Ziziphus jujuba* Mill Var *spinosa* (Bunge) Hu, on the penicillin sodium induced hyperactivity in rat CA1 neurons *in vitro*. **METHODS**: Hippocampal slices were obtained from the Sprague-Dawley rat brain and populational signals were measured from CA1 neurons of hippocampal slices using the extracellular recording technique. **RESULTS**: Penicillin sodium of 500, 1000, and 2000 kU/L were found to excite hippocampal CA1 neurons in a concentration-dependent manner *in vitro*. This excitatory effect of penicillin sodium could be inhibited by phenobarbital sodium of 0.02 - 0.05 g/L and JuA of 0.05 - 0.10 g/L. **CONCLUSION**: A high dose of JuA can inhibit the hyperactivity of hippocampal CA1 area induced by penicillin sodium. The inhibition of the amplitude of the first population spike (PS) and the latency of PS are more pronounced than the slope of the field excitatory postsynaptic potential.

INTRODUCTION

Ziziphus jujuba Mill Var *spinosa* (Bunge) Hu, has been used for decades as a sedative-hypnotic drug for symptoms caused by the hyperactivity of the central nervous system (CNS) such as insomnia and dysphoria[1]. Experimental studies validated that it could inhibit the spontaneous activity, facilitate the hypnotic action of pentobarbital, and antagonize the excitatory action of morphine and pentylenetetrazole[1,2]. Several constituents in it were found effective, ie, saponin[3,4], flavone[3,5], *etc*. Recently, several alkaloids were also observed in it[6]. However, most of previous experiments focused on the study of behavioral changes and the mechanism of its inhibitory action has not been fully understood yet.

There are many mechanisms of depressant drugs on the CNS. It is well known that many CNS depressant drugs exert their actions through the γ-aminobutyrate (GABA)-mediated mechanisms, while some experimental studies following that suggest that the activity of central neurons can also be inhibited by increasing the potassium conductance[7]. Penicillin sodium is considered as a suitable excitatory agent of the CNS since it can improve the activities of the CNS through the GABA and glutamate way[8]. For example, it facilitates excitatory synaptic events in invertebrate systems[9,10], and has an action at pre-synaptic terminals[11]. It also competes the binding action of GABA and its receptor[12].

The present study tried to examine the effect of jujuboside A (JuA)[13], a constituent of Chinese herbal medicine *Ziziphus jujuba* Mill Var *spinosa* (Bunge) Hu, on the penicillin sodium-induced hyperactivity in CA1 area of the rat hippocampal slices *in vitro*.

MATERIALS AND METHODS

Chemicals and animals JuA was provided by National Institute for the Control of Pharmaceutical and Biological Products; penicillin sodium and phenobarbital sodium from China Medical Bioproduct Co; pontamine sky blue (Chicago sky blue 6B) from Sigma Chemical Co; Sprague-Dawley rats (♂♀, Grade Ⅱ, Certificate No 2001001, weighing 180 - 200 g) were obtained from the Zhejiang Center of Laboratory Animals.

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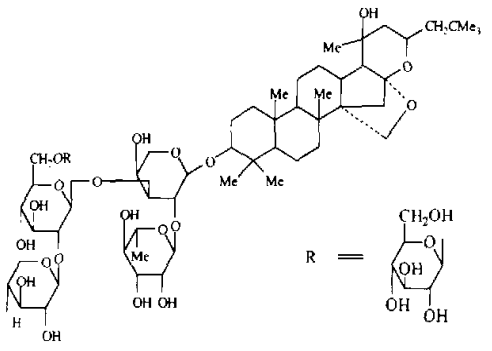

Structure of jujuboside A

Preparation of slices Rat hippocampal slices were prepared as previously described[14]. Briefly, the brain was removed from adult rat without anaesthesia, and the hippocampus was dissected free and placed in chilled artificial cerebrospinal fluid (ACSF). Transversal slices ranged from 450 to 550 μm in thickness were cut with a mechanical chopper at 0 ℃ and incubated in the ACSF at 25 ℃ for at least 90 min. The ACSF used was previously saturated with 95 % O_2 and 5 % CO_2. Before measurements, slices were transferred to an interface chamber where they were continuously perfused at 1 mL/min with the saturated ACSF. Water controlled by a heat-sensitive resistance was used to keep the bathing medium at 34 - 35 ℃ bubbled with the mixed gas of 95 % O_2 and 5 % CO_2.

Application of drugs Penicillin sodium of different concentrations dissolved in the ACSF was applied to excite the hippocampal slices. After the slices were excited by penicillin sodium, they were superfused using the control medium (normal ACSF), phenobarbital sodium, and JuA of different concentrations.

Extracellular recording Field potentials were recorded extracellularly using glass microelectrodes. The electrode was made by a micropipette puller (Model P-87, Sutter Instrument Co, USA) of 1 - 2 MΩ resistance, and filled with 0.5 mol/L sodium acetate and 2 % pontamine sky blue. It was placed in the pyramidal cell layer of CA1 region during recording. Electrical pulses of 50 μA, 0.01 Hz, and 0.1 ms duration (JJC-4, Guotai, Shanghai, China) were used to stimulate the cells. The pulse was rectified to stabilize the current before being applied to the schaffer collateral commissural pathway via a monopolar and insulated tungsten wire electrode. Both the recording and stimulating electrodes were controlled by the micromanipulators (WPI Inc, USA). Potentials were provided through a high impedance amplifier (FD223, WPI Inc, USA) to a DC preamplifier (Model HZG-81, Shanghai Jialong Inc, China).

Data analysis The signals from the preamplifier were sampled at the frequency of 10 kHz by the Powerlab/4S A/D conversion system (AD Inc, Australia). In each experiment, the slope of the field excitatory postsynaptic potential (fEPSP) and the number of population spike(PS), the amplitude of the first PS and the latency of the PS, were obtained and normalized by the mean values of the baseline recording. All values are given in $\bar{x} \pm s$. The data recorded were analyzed using the paired student t-test ($P < 0.05$).

RESULTS

The slices that could keep an induced field potential with a PS of 1 mV were selected for the experimental studies (Fig 1A). Medium perfusing periods were 10 min for penicillin sodium; and 15 min for other drugs.

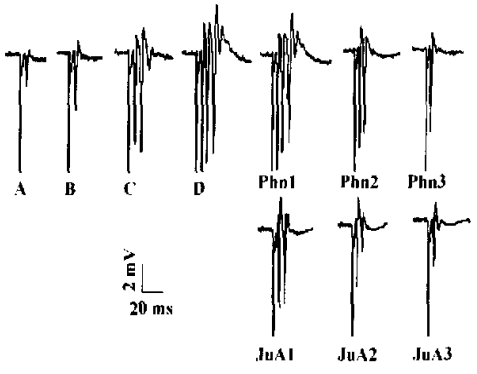

Fig 1. The field potentials of the slices treated by different drugs. A: Normal slice; B, C, and D: Slices treated with penicillin sodium 500 kU/L, 1000 kU/L, and 2000 kU/L, respecitvely. Phn1, Phn2, Phn3: Slices treated with phenobarbital sodium 0.01, 0.05, 0.10 g/L after penicillin sodium 2000 kU/L treatment (Phn: phenobarbital sodium). JuA1, JuA2, JuA3: Slice treated with JuA 0, 0.05, 0.10 g/L after penicillin sodium 1000 kU/L treatment.

Effect of penicillin sodium on rat hippocampal slices Solid penicillin sodium was dissolved in the distilled water, and diluted by the ACSF into three different doses, 500 kU/L, 1000 kU/L, and 2000 kU/L. Penicillin sodium was applied to the incubated slices

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for about 10 min and the field potentials were recorded every other minute. As a result, penicillin sodium excited the normal hippocampal slices. When penicillin sodium 1000 kU/L and 2000 kU/L were applied, the number of the PS and the amplitude of the first PS were increased markedly (Tab 1). However, when penicillin sodium of 500 kU/L was applied, there was no significant increase in both the number of PS and the amplitude of the first PS compared with control ($P > 0.05$, Tab 1). The responses of the slices after 10 min bathing of penicillin sodium of different concentrations were shown in Fig 1.

Tab 1. The concentration-dependent relationship of the excitatory effect of penicillin sodium on hippocampal slices and the inhibitory effect of phenobarbital sodium on the excitatory status induced by penicillin sodium 2000 kU/L. $n = 14$. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* control. $^eP < 0.05$ *vs* phenobarbital sodium 0 g/L group.

Dose of drugs		Number of PS	Amplitude of PS/mV
Control(ACSF)		1.02±0.14	2.1±0.3
Penicillin sodium/kU·L^{-1}	500	2.2±0.4	3.4±0.7
	1000	3.8±0.7^b	4.5±0.9^b
	2000	5.5±1.0^c	6.3±1.4^c
Phenobarbital sodium/g·L^{-1}	0	4.9±0.4	6.28±0.11
	0.01	3.5±0.3	6.21±0.10
	0.02	2.6±0.3^e	5.95±0.16
	0.05	2.01±0.19^e	3.29±0.16^e

PS: population spike.

Effect of phenobarbital sodium on penicillin sodium-induced hyperactivities Slices excited by penicillin sodium of 2000 kU/L were used, both the number of PS and the amplitude of the first PS increased significantly compared with the control ($P < 0.01$, Tab 1). Solid phenobarbital sodium was dissolved in the distilled water, and diluted by the ACSF into different concentrations (0.01 g/L, 0.02 g/L, and 0.05 g/L) before applied to the penicillin sodium pre-incubated slices. Phenobarbital sodium of 0.01 g/L had no significant effect ($P > 0.05$, Tab 1). Phenobarbital sodium of 0.02 g/L inhibited the number of PS ($P < 0.05$), but no effect on the amplitude of the first PS ($P > 0.05$, Tab 1). Phenobarbital sodium of 0.05 g/L inhibited both the number of PS and the amplitude of the first PS ($P < 0.05$, Tab 1). The responses of the slices treated with different concentrations of phenobarbital sodium for 15 min were shown in Fig 1.

Effect of JuA on penicillin sodium-induced hyperactivities Slices excited by penicillin sodium of 1000 kU/L were used, both the number of PS and the amplitude of the first PS were increased greatly compared with the control, but it had no significant influence on the latency and the slope of the fEPSP ($P > 0.05$, Tab 2). Solid JuA was dissolved in distilled water, and diluted by the ACSF into different concentrations (0.01 g/L, 0.05 g/L, and 0.10 g/L) before applied to the penicillin sodium pre-incubated slices. JuA of 0.01 g/L had no significant effect ($P > 0.05$, Tab 2). Statistically, JuA 0.05 g/L can inhibit both the number of PS and the amplitude of the first PS, but it had no significant influence on the latency and the slope of the fEPSP ($P > 0.05$, Tab 2). JuA of 0.10 g/L inhibited both the number of PS and the amplitude of the first PS ($P < 0.01$, Tab 2). In addition, it also prolonged the latency and decreased the slope of the fEPSP ($P < 0.01$ or 0.05) (Tab 2). The slope of the fEPSP was more difficult to be influenced by the treatment of drugs than other parameters of the potential. The responses of the slices after 15 min treatment of JuA of different concentrations were shown in Fig 1.

Tab 2. The inhibitory effect of different concentration of JuA on the excitatory status induced by penicillin sodium 1000 kU/L. $n = 10$. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* control. $^eP < 0.05$, $^fP < 0.01$ *vs* penicillin sodium 1000 kU/L.

Concentrations of drugs		Number of PS	Amplitude of first PS/mV	Latency of PS/ms	Slope of fEPSP
Control		1.02±0.14	2.1±0.3	5.7±0.8	1.3±0.7
Penicillin sodium/kU·L^{-1}	1000	4.8±0.9^b	4.2±1.3^c	5.0±1.0	1.4±0.3
JuA/g·L^{-1}	0.01	4.67±0.13	4.5±1.0	4.5±0.6	1.5±0.6
	0.05	3.2±0.6^e	2.8±1.3^f	4.8±1.0	1.4±0.5
	0.10	1.75±0.23^f	1.9±0.6^f	7.0±1.1^f	1.10±0.20^e

PS: population spike; fEPSP: field excitatory post-synaptic potential.

DISCUSSION

We have studied that the effect of penicillin sodium on hippocampal slices and the effect of phenobarbital sodium and JuA on penicillin sodium-induced hyperactivity in hippocampal slices.

It was found that penicillin sodium could induce orthodromic hyperactivity in hippocampal slices. The amplitude and number of PS increased greatly but the fEPSP were not greatly affected even when the concentration was increased. Furthermore, we have also found that phenobarbital sodium of 0.05 g/L, commonly used in practice, could inhibit the hyperactivity induced by penicillin sodium. Here phenobarbital sodium acts as a positive control that has inhibitory effect on the CNS activity. When different concentrations of JuA were added to the hippocampal slices excited by penicillin sodium of 1000 kU/L, statistical data showed that several parameters enhanced by penicillin sodium were all inhibited to a certain degree and the effect was concentration-dependent. A high dose of JuA (0.10 g/L) could inhibit the hyperactivity completely and quickly. It took a longer time for the slices to recover from the inhibited situation. The middle concentration of JuA (0.05 g/L) could only reduce the number of PS and the amplitude of the first PS. However, the fEPSP and the latency of the PS were not affected at all. As the concentration was reduced to 0.01 g/L, little effect of JuA was found on the penicillin sodium-induced hyperactivity. Because JuA can bind to CaM in different manners and the binding is concentration-dependent, it may exert some effect through inhibiting the post-synaptic reactions induced by penicillin sodium[13]. Our results proved that the high concentration of JuA extracted from *Ziziphus jujuba* Mill Var *spinosa* (Bunge) Hu was very effective in inhibiting the penicillin sodium-induced hyperactivity in hippocampal slices *in vitro*. It should be noted that JuA is only one component of the seed *Ziziphus jujuba* Mill Var *spinosa* (Bunge) Hu, which was tested in this study. Further study on the combination of the different components of this seed will help reveal the balanced effect of the complex Chinese traditional medicine[15,16].

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酸枣仁皂甙 A 对青霉素钠诱发大鼠海马 CA1 区过度兴奋的抑制作用[1]

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关键词 酸枣仁皂甙 A；海马；神经抑制；GABA

目的：观察中药酸枣仁皂甙 A 对青霉素钠诱导产生的大鼠海马脑片 CA1 区兴奋性放电的抑制作用。

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方法：细胞外记录离体大鼠海马脑片 CA1 区锥体细胞层群体峰电位. **结果**：青霉素钠 500、1000 和 2000 kU/L 可剂量依赖地诱导海马脑片上 CA1 区神经元的兴奋. 苯巴比妥钠 0.02－0.05 g/L 和酸枣仁皂甙 A 0.05－0.10 g/L 都可以剂量依赖性地抑制这种青霉素钠诱发的兴奋反应. **结论**：高剂量的酸枣仁皂甙 A 能够抑制青霉素钠诱导的海马 CA1 区兴奋性电位. 群峰电位(PS)的个数和第一个峰电位的幅度受到的抑制较明显，而兴奋性突触后场电位的变化不大.

（责任编辑 吴民淑）

关于申报评选 2002 年第六届 中国药理学会 Servier 青年药理学工作者奖的通知

中国药理学会经与法国 Servier 研究院商定，第六届中国药理学会 Servier 青年药理工作者奖将于 2001 年 12 月底评出. 现将有关事宜通知如下：

一. 获奖候选人条件：

1. 2001 年底以前入会的中国药理学会会员，(老会员已交纳 2001－2003 年会费的)；

2. 年龄在 37 岁以下(1965.3.1 以后出生)；

3. 在国内从事药理学研究并取得优秀成绩，不包括在国外做过的工作；

4. 在国内工作至少 2 年以上(硕士、博士生在读学位前有 2 年以上工作经历也可以)；

5. 报奖者的两名推荐专家必须是中国药理学会会员；

6. 提交近三年发表与未发表的研究内容写成的论文(主要在国内完成)参加评选.

二. 报名及评选程序：

符合上述条件的青年药理学工作者接到通知后，将报奖材料统一送到学会办公室，由学会评奖委员会评选. 报名时需报以下材料：(1)按要求填写申报表(申报表可在中国药理学会网页上查找或向学会办公室索取)；(2)论文一篇，全文用中文和英文撰写(用 16kB5 纸打印)并附软盘(Word 格式或文本文件). (3)两位药理学会专家(教授或相当职称者)的推荐书，材料不符合标准及弄虚作假取消评审资格.

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