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The effect of Jujuboside A on the evoked field potentials of granule cells in dentate gyrus*

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Abstract: Jujuboside A (JuA) is a main component of Jujubogenin extracted from the seeds of Ziziphus. The authors have not seen any report on JuA's direct effect on the neurons of the central nervous system. This study aimed to assess the effect of JuA on paired-pulse responses of dentate gyrus granule cells in urethane-anaesthetized rats, used intracerebroventricular (i.c.v.) JuA to mimic in vitro bath conditions in vivo. Paired-pulse stimuli with 80ms interpulse interval were used to stimulate the perforant pathway. Evoked responses were recorded in the dentate gyrus cell layer after i.c.v. administration of 0.9% normal saline or JuA. In the first responses, the slopes of excitatory postsynaptic potential (EPSP1) and the amplitudes of population spike (PS1) decreased significantly after administration of JuA while the PS1 latencies increased significantly. In the second responses, the EPSP2 slopes and PS2 latencies were changed similarly to those of the first ones, but PS2 amplitudes increased. The results showed that JuA may have some inhibitory effect on the granule cell excitability mediated by presynaptic mechanism but may have little effect on the excitability mediated by postsynaptic mechanism since the second evoked N-methyl-D-aspartic mediating paired-pulse facilitation is a postsynaptic mechanism.

Key words: Field potential, Dentate gyrus, Jujuboside A(JuA), Inhibitory effect
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INTRODUCTION

The seed of Ziziphus Spinosa has long been known in Chinese medical practice (Ying, 1993) as having sedative and hypnotive effect on the central nervous system (CNS). JuA is a main component of jujubogenin extracted from the seeds of Ziziphus. Some reports showed that JuA is a noncompetitive inhibitor of calmodulin (Zhou 1994). Wu SX et al.(1993) studied the effects of JuA on the CNS activities of mouse. However, there is apparently no report showing the direct effect of JuA on the CNS neurons. In this work we tested the inhibitory effect of JuA on the evoked field potentials of granule cells in the dentate gyrus by i.c.v. administration of JuA. The hippocampus and the dentate gyrus (DG) are two main parts of the highly laminated hippocampal formation structure. DG has well-defined afferents (perforant pathway, PP) and efferents (mossy fiber, MF) (Andersen et al. 1971); and yields large amplitude, stable, and easy to measure evoked field responses elicited from activation of the PP. Because the dentate gyrus is just situated in the lateral ventricle, the drug injected into the brain ventricle will reach the dentate gyrus and affect directly the activities of its main neurons, namely granule cells.

METHODS

Adult Sprague-Dawley rats (approximately 250g) were anaesthetized with urethane (1.25 g/kg) and placed in a stereotaxic apparatus. A bipolar stimulating electrode and a monopolar recording electrode, made from insulated nichrome wires (80μm diameter), were inserted

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into the angular bundle of the perforant pathway (AP −7.1, ML 4.2, DV 3.2) and the dentate gyrus granule cell layer (AP −3.5, ML 2.0, DV 2.0), respectively. The final depth placements of the electrodes were selected according to the electrophysiological guidance (Gilbert et al. 1999; John et al. 1999; Paul et al. 1998). A stainless steel wire was fixed with a screw over the contralateral cerebellum (AP −10.0, ML 3.0) served as reference electrode. A plastic guide cannula was inserted into the lateral ventricle (AP −0.8, ML 1.5, DV 3.5) for i.c.v. injection. After the fixing of the electrodes, it took about 2 hours for the evoked responses to stabilize before the start of the tests.

Paired-pulse stimuli were delivered by a A360D constant current stimulus isolator (WPI Inc.) at a frequency of 1 pulse pair every 30 seconds. The pulse duration was 0.1 ms. The interpulse interval was 80ms that would produce the effect of paired-pulse facilitation in the dentate gyrus (Joy, 1993). The stimulus intensity was selected at a level of producing a PS1 amplitude of about 80% of the maximal response for each rat. The evoked field responses were amplified and digitized at 10 kHz by a PowerLab system (AD Instruments) and were stored in a computer.

JuA provided by the Chinese Drug and Biological Standardization Institute was dissolved in normal saline. Every test rat was given three times of i.c.v. injections according with the experimental procedures in Albertson's(1989) paper. The first injection was 3 μl of saline. The second and the third injections were 3 μl of JuA (30 mg/ml). The injection intervals were 30 minutes. Baseline of paired-pulse responses was recorded for 20 minutes before any drug administrations. Then, the responses were recorded during 10−30 min after each i.c.v. injection.

The values of each response, including the slope of the excitatory postsynaptic potential (EPSP), the amplitude of the population spike (PS) and the PS latency (PSL) were computed and normalized by the mean values of baseline recording. The differences in values between the data recorded after saline administration and those after the two JuA administrations were analyzed by paired Student's t-test.

RESULTS

Fig. 1(a) shows the responses evoked by a paired-pulse stimulus after i.c.v. injection of normal saline. The effect of paired-pulse facilitation of PS2 was evident since the second population spike (PS2) was much larger than the first one (PS1). After the injection of JuA, PS1 was

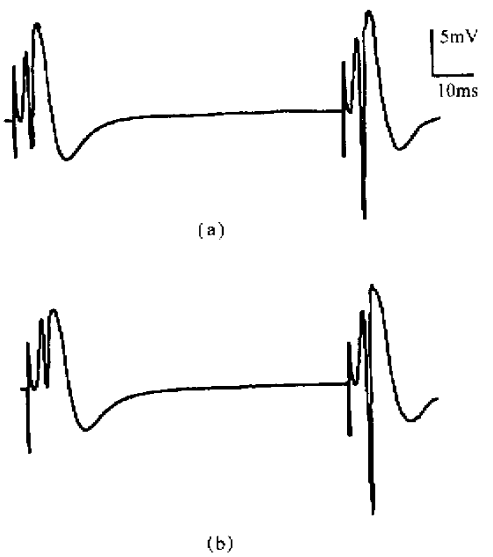

Fig. 1 Example of responses after normal saline injection (a) and JuA injection (b)

depressed but PS2 was not, as shown in Fig. 1 (b). Table 1 shows the changes of evoked responses after injections of saline or JuA. After the injection of saline, the evoked responses were not changed much or had a slightly up shifting of PS and EPSP compared to the baseline recording, and were similar to the responses reported by Gilbert (1999) and John (1999). After the first injection of JuA, both EPSP1 and PS1 decreased significantly. After the second injection of JuA, compared to the values obtained after the injection of saline, the EPSP1 and PS1 decreased further by 14.1% ($P<0.01$) and 20.5% ($P<0.05$), respectively. EPSP2 decreased 9.4% ($P<0.01$). The increases in PS1 latency (PSL1) of 17.7% ($P<0.01$) and PS2 latency (PSL2) of 10.1% ($P<0.01$) were significant, too. PS2 also increased by 13.8% ($P<0.01$).

Table 1 Changes in evoked responses of paired-pulse stimuli after injections of normal saline and JuA (mean ± SD, $n = 5$)

	EPSP1 (%)	PS1 (%)	PSL1 (%)	EPSP2 (%)	PS2 (%)	PSL2 (%)
Normal saline	99.3 ±0.6	106.9 ±10.1	100.2 ±1.8	100.3 ±0.6	103.7 ±3.5	99.0 ±0.9
The first JuA	94.6 ±3.5*	89.8 ±11.1*	105.3 ±4.2	100.4 ±2.1	106.7 ±2.5	103.6 ±3.5*
The second JuA	85.2 ±4.2**	86.4 ±10.5*	117.9 ±7.2**	90.9 ±5.0**	117.5 ±3.3**	109.1 ±4.3**

* $P<0.05$, ** $P<0.01$ v. normal saline.

DISCUSSION

When the granule cells in the dentate gyrus were inhibited, the EPSP and PS of evoked responses would generally decrease while PS latency would increase. In the present experiment, after two times injections of JuA, EPSP1 and PS1 of the first responses decreased significantly while PS1 latency increased. The data indicated that the excitability of granule cells was inhibited by JuA. For the second responses, although EPSP2 decreased and PS2 latency increased as indications of inhibitory effects, PS2 increased. The paired-pulse facilitation of PS2 is considered to result from a selective enhancement of N-methyl-D-aspartic (NMDA) mediated synaptic current that has a slower onset and a longer duration than non-NMDA-mediated current (Joy et al., 1993). It is a non-presynaptic mechanism. JuA may have inhibitory effect on the excitability of granule cells mediated by presynaptic mechanism but may have little effect on the excitability mediated by postsynaptic mechanism. In addition, after administration of JuA some of the neurons which failed to fire by the first stimulus because of the excitability decrease, were fired by the second stimulus. These resulted in the larger PS2.

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