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Effects of lithium on the synaptic plasticity in dentate gyrus region of rat hippocampus

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Abstract: AIM To investigate the mechanism of therapeutic action of lithium with respect to long-term potentiation(LTP) elicited in dentate gyrus(DG) region of rat hippocampus. **METHODS** To use conventional extracellular recording technique in hippocampal slices *in vitro*. **RESULTS** Lithium was found to reversibly increase excitatory postsynaptic potentials in the DG region of rat hippocampus. Under control conditions, titanic stimulation (100 Hz, 1 s) of medial perforans pathway induced LTP. Acute treatment of low concentration lithium (2, 6 mmol·L^{-1}) did not affect the LTP induced by titanic stimulation, while its higher concentration (10 mmol·L^{-1}) inhibited the amplitude of LTP in the DG region of rat hippocampus. Furthermore, lithium treatment (10 mmol·L^{-1}) decreased paired-pulse facilitation (PPF) measured at 50 ms inter-pulse interval while, at lower concentrations, lithium treatments (2, 6 mmol·L^{-1}) did not affect PPF significantly. We also found that the effects of lithium (10 mmol·L^{-1}) on PPF were different at different $[Ca^{2+}]_o$. **CONCLUSION** Lithium can inhibit the LTP magnitude in rat hippocampus probably through presynaptic mechanisms. These alterations of neurophysiological responses may be related to the therapeutic action of lithium salts in mania and depression as well as producing side effects of lithium chemotherapy.

Key words: lithium; hippocampus; potentiometry, long-term potentiation; paired-pulse facilitation

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Lithium has been widely used as an effective antidepressant in the treatment of manic depressive disorders[1]. At present, lithium is the standard prophylactic agent for bipolar disorder and is taken by 1% of the population in Western countries[2]. It is believed that two lithium-sensitive signal transduction pathways are active in the brain; these are mediated by glycogen synthase kinase 3β[3] and inositol (1, 4, 5)-trisphosphate[4] signaling. Despite numerous biochemical studies on the effects of acute and chronic lithium treatment, little is known about the mechanisms that lithium can modify neurotransmission within the CNS[5,6]. Colino, *et al*[7] have observed that lithium enhanced the excitatory postsynaptic potentials (EPSP) in the CA1 region of hippocampus slices probably through presynaptic mechanisms[8]. Protein kinase C was found to be involved in the increase in EPSPs[9]. Ballyk, *et al*[10] have reported that lithium inhibited the induction and maintenance of long-term potentiation(LTP), which represents the cellular substrate for long term behavioral changes and may be a possible target of different psychoactive drugs, at stratum radiatum-CA1 synapses. Until now, however, there have been no reports regarding the effects of lithium on LTP elicited in the dentate gyrus(DG) region. In the present study, we thus investigated the effects of lithium on LTP elicited in DG region of acutely prepared hippocampal slices.

1 MATERIALS AND METHODS

Brain slices of the hippocampus region of 27- to 30-day-old Wistar rats were obtained by using standard procedures[11,12]. Slices were kept at least 1 h at room temperature in a holding chamber

containing artificial cerebrospinal fluid (ACSF) bubbled with 95% O_2 and 5% CO_2. Slices were transferred to a recording chamber that was continually superfused with ACSF at 1.5 mL·min^{-1}. The ACSF contained (mmol·L^{-1}): NaCl 124, KCl 5, NaH_2PO_4 1.25, $MgCl_2$ 1.25, $NaHCO_3$ 26, $CaCl_2$ 2.0. Glass recording electrodes (1 – 2 MΩ) were filled with ACSF and inserted into the cell body layer of DG granule cells to record field EPSPs. Bipolar tungsten microelectrodes were inserted into the medial perforans pathway. In addition, stimulating electrodes were placed as far away from recording sites as possible to avoid evoking monosynaptic GABA-ergic activities due to the direct stimulation of interneurons.

A typical experiment began with the establishment of an input-output (I-O) curve that included stimulation intensities evoking threshold, 30% maximal, 50% maximal and maximal field EPSP amplitudes. Slices exhibiting a maximal field EPSP amplitude < 2.0 mV, signs of hyperexcitability, or unstable baseline potentials (drift of response > 10% over the 20 min baseline recording period) were discarded. Stimulus intensity was adjusted to evoke a field EPSP of approximately half of the maximal amplitude. For additional paired-pulse facilitation protocols, the field EPSP amplitude was adjusted to the original control values. LTP was induced by applying a 1 s train of 100 Hz high frequency stimulation (HFS). At the end of each experiment, the I-O curve was reconstructed. In the experiments using acute lithium treatment, the slices were incubated for 3 – 4 h before the tetanus in the saline containing lithium (2, 6, 10 mmol·L^{-1}). For statistical analysis of field EPSP, responses were first collected and averaged in 10 min blocks: 20 min for the baseline and 40 min after tetanus. The field EPSP slopes (millivolts per millisecond) were calculated as the initial slopes of field EPSPs by using least-squares regression, and data for each experiment were normalized relative to baseline. Slices from a single animal that received the same treatment were averaged together and represented an $n = 1$. When comparing results from different slices, a one-way ANOVA or multiple two-tailed unpaired t tests were performed at the same time intervals. Data were presented as $\bar{x} \pm s$ and were collected and averaged in 5 min blocks for graphic representations. Probabilities less than 0.05 were considered significant.

2 RESULTS

2.1 Lithium superfusion reversibly increases excitatory postsynaptic potentials slope

To test whether lithium could affect normal synaptic transmission, various concentrations of lithium (2, 6, 10 mmol·L^{-1}) were superfused onto hippocampus slices(Fig 1), and field EPSPs were recorded in the DG region(Tab 1). After recording a stable baseline for at least 20 min, lithium was continuously superfused onto the slice for about 20 min. In accordance with other reports[13,7,8], lithium reversibly increased the field EPSP slope recorded in the DG region and this potentiation began at about 5 min after the application of the solution and disappeared about 15 min after washout. The peak potentiation appeared at about 40 min.

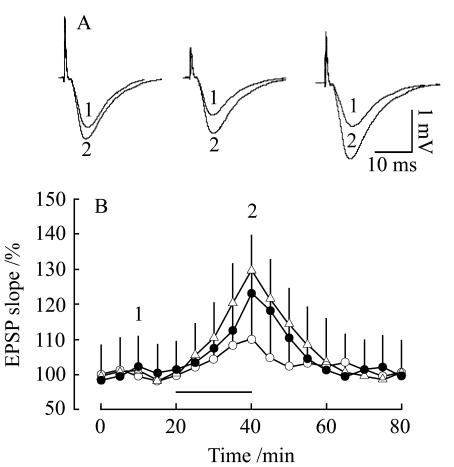

Fig 1. Effect of lithium on field excitatory postsynaptic potentials (EPSP) in dentate gyrus (DG) region. A: represents single sweep of extracellular recorded field potentials. Calibration is 1 mV/10 ms. B: the dark bar above the graph indicates the time at which drug application occurred. (○): 2 mmol·L^{-1} ($n = 6$), (●): 6 mmol·L^{-1} ($n = 8$), (△): 10 mmol·L^{-1} ($n = 6$) lithium, respectively. $\bar{x} \pm s$.

Tab 1. Effect of lithium(Li) superfusion on EPSP slope

Li/mmol·L^{-1}	n	EPSP slope/%
2	6	111 ± 7
6	8	120 ± 6
10	6	128 ± 6

After recording EPSPs for at least 20 min, lithium of different concentration was superfused onto brain slices for about 20 min.

2.2 Lithium treatment inhibits the expression of long-term potentiation

To investigate the acute effect of lithium on LTP elicited in the DG region, after kept incubation in the lithium-containing solution, the slice was tetanized. LTP was induced by using one train of 100 pulses delivered at 100 Hz, recorded for 60 min. Averaged field EPSP slopes were plotted as percentage of baseline response. Recordings in the DG region showed that the EPSP amplitude was significantly increased 40 min after the administration of HFS in control hippocampal slice preparations [$n = 13$, $t(12) = 3.92$, $P < 0.05$], while in the acute lithium treatment (10 mmol·L^{-1}) hippocampus slices, the EPSP amplitude was unaffected significantly increased 40 min after the administration of HFS [$n = 15$, $t(14) = 2.03$, $P > 0.05$]. Moreover, the groups were significantly different from each other [$F(1,26) = 4.67$, $P < 0.05$], indicating that acute lithium treatment (10 mmol·L^{-1}) inhibited the expression of LTP elicited in the DG region of hippocampus (Fig 2). It is noteworthy that acute lithium treatment at lower concentrations (2 mmol·L^{-1}, 6 mmol·L^{-1}) did not affected LTP expression of the DG region of hippocampus (Tab 2).

2.3 Lithium treatment inhibits paired-pulse facilitation in hippocampal dentate pyrus region

To determine whether lithium might affect the presynaptic location at the tested concentrations, we studied PPF at 50 ms interstimulus interval (Tab 3). PPF is a presynaptic facilitation revealed by the second of a pair of stimulation pulses, delivered at short intervals. It is widely believed that PPF is an efficient test to detect changes within

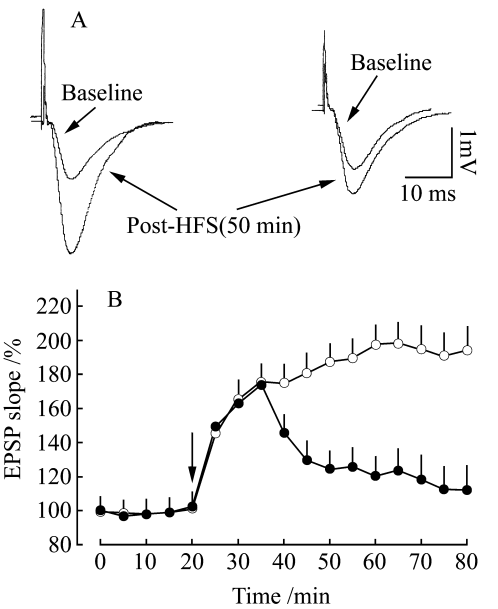

Fig 2. Effect of acute lithium treatment on the long-term potentiation(LTP) of extracellular recorded field EPSP. A: represents single sweep of extracellular recorded field potentials in control conditions and lithium treatment conditions. Calibration is 1 mV/10 ms. B: shows the LTP in control conditions [●, (195 ± 14)%, $n = 13$] and in lithium-bathed slices [○, Li 10 mmol·L^{-1}, (118 ± 16)%, $n = 15$]. The induction of LTP is unaffected by lithium (the arrow depicts high frequency stimulation), whereas the following stage of LTP is reduced. $\bar{x} \pm s$. $^{*}P < 0.05$, compared with control group.

Tab 2. Effects of Li treatment on LTP expression

Li/mmol·L^{-1}	n	EPSP slope/%
0	13	195 ± 12
2	8	191 ± 14
6	10	190 ± 13
10	15	119 ± 9*

The slices were incubated for 3 – 4 h before the tetanus in the saline containing lithium (2, 6, 10 mmol·L^{-1}). $\bar{x} \pm s$. $^{*}P < 0.05$, compared with 0 mmol·L^{-1} group.

Tab 3. Effect of Li treatment on paired-pulse facilitation(PPF) measured at 50 ms inter-pulse interval

Li/mmol·L^{-1}	n	PPF (% of EPSP2 slope/EPSP1 slope)
0	8	161 ± 10
2	10	158 ± 12
6	7	150 ± 13
10	12	121 ± 12*

See Tab 2 for the treatment. $\bar{x} \pm s$. $^{*}P < 0.05$, compared with 0 mmol·L^{-1} group.

presynaptic terminals[14-16]. The magnitude of PPF was estimated before and after lithium superfusion by plotting the slope of the field EPSP induced by the second of a pair of evoked potentials as a percentage of the slope of the first field EPSP as a function of the inter-pulse intervals (milliseconds) between the two stimuli. Stimulation intensity was set to evoke about 50% of the maximal field EPSP slope. The magnitude of PPF measured at the 50 ms inter-pulse interval was significantly reduced 10 mmol·L^{-1} lithium treatment, not affected by 2 mmol·L^{-1} and 6 mmol·L^{-1} lithium treatments. It is interesting to note that lithium treatment (10 mmol·L^{-1}) also affected PPF measured at other inter-pulse intervals (Fig 3).

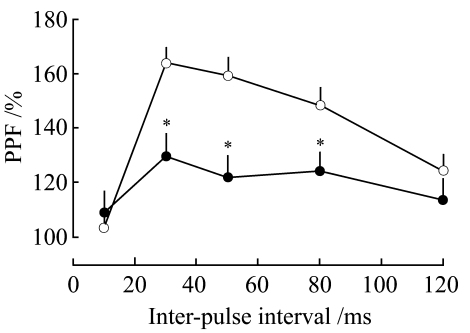

Fig 3. Paired-pulse facilitation is affected by lithium. PPF measured at different inter-pulse intervals is altered by 10 mmol·L^{-1} lithium treatment. (○): control ($n = 8$), (●): acute lithium (10 mmol·L^{-1}, $n = 12$). $\bar{x} \pm s$. $^{*}P < 0.05$, compared with control group.

2.4 Lithium has different effects on paired-pulse facilitation in different extracellular calcium concentration ($[Ca^{2+}]_o$)

The effects of changing the $[Ca^{2+}]_o$ on PPF in control and lithium-treated slices were also examined. The PPF was measured at 50 ms inter-pulse interval, which exhibited the largest effect of lithium in normal $[Ca^{2+}]_o$ (see Fig 3). In control slices, lowering $[Ca^{2+}]_o$ to 0.5 mmol·L^{-1} decreased whereas elevating to 5 mmol·L^{-1} increased the first EPSP of the pair, leading to an increase and decrease in PPF, respectively (Fig 4). Lithium decreased PPF in all three $[Ca^{2+}]_o$. However, the effect of lithium in 0.5 mmol·L^{-1} $[Ca^{2+}]_o$ was relatively small and not statistically significant (Fig 4). The percentage of lithium effect under different $[Ca^{2+}]_o$ could be calculated by (control-lithium)/control. The results were as follows: $[Ca^{2+}]_o$ 0.5 mmol·L^{-1} 6%; $[Ca^{2+}]_o$ 2 mmol·L^{-1} 24%; $[Ca^{2+}]_o$ 5 mmol·L^{-1} 28%. Treatment with lithium became more effective when combined with increasing $[Ca^{2+}]_o$. Thus, the effect of lithium at decreasing PPF was slightly smaller when release probability was reduced by lowering $[Ca^{2+}]_o$ and was significantly larger when release probability was increased by elevating $[Ca^{2+}]_o$. Since changing $[Ca^{2+}]_o$ is known to alter transmitter release, modulating the effectiveness of changing $[Ca^{2+}]_o$ with lithium supports the notion that lithium may modulate transmitter release mechanisms.

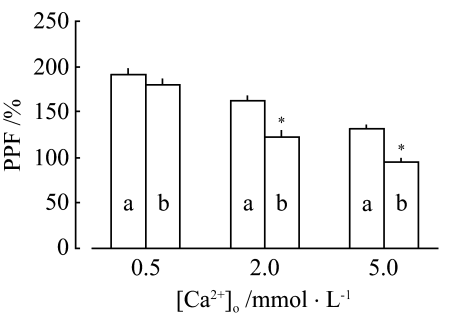

Fig 4. Effect of lithium on paired-pulse facilitation at a 50 ms inter-pulse interval at different $[Ca^{2+}]_o$. a: control($n = 5$), b: lithium (10 mmol·L^{-1}) treated($n = 6$). $\bar{x} \pm s$. $^{*}P < 0.05$, compared with control group.

3 DISCUSSION

In accordance with other studies[5,6,10], lithium superfusion reversibly increases synaptic transmission in the hippocampal DG region. Lithium has been reported to inhibit the function of glutamate transport in presynaptic terminals, leading to the enhancement of glutamate in the synaptic cleft and the neuronal excitability[17]. Lithium has also been suggested to broaden action potentials[7]. So, the lithium-induced increment of presynaptic calcium may account for the synaptic enhancement. We also investigate the effects of acute lithium treatment on LTP elicited in the hippocampal DG region. Induction of LTP requires postsy-

naptic activation of the *N*-methyl-*D*-aspartate (NMDA) receptor[18] and a subsequent intracellular increase in calcium concentration[19]. Maintenance of LTP requires both presynaptic mechanisms such as enhanced transmitter release from nerve terminals[20], and postsynaptic mechanisms such as activation of Ca^{2+}/calmodulin kinase Ⅱ phosphorylation of functional proteins[21,22]. The blockade of LTP elicited in the hippocampal DG region is a novel finding. It has been shown that lithium is a potent and selective inhibitor of glycogen synthase kinase 3β[3]. It is conceivable that NMDA receptor subunits are a target for glycogen synthase kinase 3β and that lithium treatment suppresses NMDA receptor functions through modulation of the receptor phosphorylation state mediated by this kinase. It is also possible that lithium disrupts the function or cycle of AMPA receptor, thus inhibiting LTP elicited in the DG region of the hippocampus. Our study supports the hypothesis that the lithium-induced inhibition of LTP is, at least in part, due to a change in presynaptic axonal excitability since PPF is decreased after lithium treatment, although an additional postsynaptic mechanism cannot be excluded. We show that lithium-treatment group exhibits smaller PPF, especially at 50 ms inter-pulse interval. Because PPF is thought to result from higher release probability to the second pulse attributable to residual presynaptic $[Ca^{2+}]_i$ left from the first pulse, a decrease in the PPF after lithium application suggests that acute lithium treatment regulate $[Ca^{2+}]_i$ dynamics within presynaptic terminals, leading to an alteration in transmitter release. It is important to note that the concentration of lithium (10 mmol·L^{-1}) required in the acute experiments to inhibit LTP expression and PPF in the DG region is much higher than the concentrations that are needed as therapeutic levels (0.5 – 1.5 mmol·L^{-1}) in humans[23]. In psychiatric chemotherapy lithium is administered daily for months or years. Whether the lithium-induced inhibition of LTP elicited in the hippocampal DG region could contribute to the therapeutic action of lithium salts in mania and depression as well as producing the side effects of lithium chemotherapy is under further research.

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锂对大鼠海马齿状回区神经元突触可塑性的影响

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摘要：目的　从齿状回长时程增强效应(LTP)方面研究锂的治疗作用机理。**方法**　细胞外记录离体海马脑片神经元兴奋性突触后电位(EPSP)。**结果**　锂可逆地增强 EPSP 的幅度。高频刺激（100 Hz，1 s)对照组大鼠海马穿通纤维，在海马齿状回(DG)区记录的 EPSP 幅度会持续增高，可以诱导出明显的突触后 LTP。若用 10 mmol·L^{-1}锂处理大鼠海马脑片，则诱导的 LTP 幅度明显降低，但低浓度锂(2，6 mmol·L^{-1})不影响 LTP 的幅度；10 mmol·L^{-1}锂明显抑制海马脑片 DG 区的脉冲间隔(IPI)为 50 ms 的双脉冲易化效应(PPF)，而低浓度锂(2，6 mmol·L^{-1})处理则不影响 PPF(IPI，50 ms)；在不同的细胞外钙浓度下，用 10 mmol·L^{-1}锂处理过的海马脑片 PPF 受到的抑制程度不同。**结论**　锂可能通过突触前的机理来抑制海马 DG 区 LTP 的幅度，这种抑制效应与锂的临床治疗狂躁症及其副作用之间的关系尚需进一步的研究。

关键词：锂；海马；电位测定法，长时程增强；双脉冲易化

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