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Lithium Carbonate Modulation of Delayed Rectifier Potassium Channel Involves Protein Kinase C/Mitogen-activated Protein Kinase Signaling in Hippocampus of Rats*

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Abstract Lithium carbonate could be used to treat or prevent brain damage following traumatic injury and neurodegenerative diseases. It has been shown that its protective effect is related to protein kinase C (PKC) and extracellular signal-related kinase (ERK). It was demonstrated that PDBu, a PKC activator, inhibited amplitudes of delayed rectifier potassium current (I_K) and produced a hyperpolarizing shift in the activation-voltage curve. The responses to PDBu were inhibited by lithium carbonate (50 μmol/L). Further studies showed that when pretreated with MEK/ERK inhibitor U0126 (20 μmol/L), although PDBu significantly reduced I_K, lithium did not reverse the effect of PDBu. Thus, the results suggested that PKC signaling cascades, along with MAPK (mitogen-activated protein kinase) pathway, were required in the phosphorylation of potassium channel, which was presented by regulation of potassium channel characteristic. AC-cAMP and their cross-talk with GC-cGMP pathway could also modulate the effect of lithium on PKC activation, which could be one of underlying mechanisms likely related to neuroprotective effect of lithium.

Key words hippocampus, lithium carbonate, delayed rectifier potassium currents, PKC, ERK

Lithium has emerged as a neuroprotective agent efficacious in preventing apoptosis-dependent cellular death [1]. Beyond its present use in bipolar mood disorder, it could be used to treat or prevent brain damage following traumatic injury such as stroke[2] and neurodegenerative diseases such as Huntington′s and Alzheimer′s diseases[3, 4]. Lithium increases cell survival by inducing brain-derived neurotrophic factor and thereby stimulating activity in anti-apoptotic pathways, including the phosphatidylinositol 3-kinase/Akt and the mitogen-activated protein kinase pathways[1]. It has been demonstrated that some of initial actions of lithium may initiate a cascade of secondary changes in the PKC signaling pathway and gene expression in the central nervous system (CNS)[5].

PKC is a ubiquitous enzyme, highly enriched in the brain, where it plays a significant role in regulating both pre- and postsynaptic aspects of neurotransmission. Biochemical, behavioral and electrophysiological data indicate that high level of PKC activity in prefrontal cortex, as seen for example during stress exposure, markedly impairs behavioral and electrophysiological measures of working memory [6]. PKC and PKC signaling appear to be a target of lithium. It was reported that chronic lithium treatment decreases the level of PKC isozymes α and ε in cell culture and in treated rodents [5].

However, the downstream regulatory effect of lithium on PKC and its interaction with ion channel remains unknown. In particular, strong evidence implicates protein kinase C (PKC) and the extracellular signal-regulated kinases (ERK) in the establishment and/or maintenance of central sensitization [7], and further the study might find regulation target of lithium from the phenomenon associated with neuronal excitability. Accumulating

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evidence suggests that the MAPK/ERK (mitogen-activated protein kinase/ extracellular signal-related kinase) plays an important and essential role in induction and maintenance of certain types of neuronal plasticity and learning[8, 9], which is downstream of both PKA and PKC, and leads to CREB protein phosphorylation in the hippocampus, as it has been known that ion channels are involved in neuronal plasticity along with changes of neuronal excitability.

Importantly, increase in neuronal excitability frequently results from phosphorylation-dependent modulation of ion channels. Voltage-gated potassium (K^+) channels are critical determinants of neuronal excitability in the central nervous system (CNS). K_v is considered as a pivotal K^+ channel determining the excitability by controlling the activity of Ca^{2+} channels in membranes and the concentration of intracellular Ca^{2+}. Besides depolarization of cell membrane, several intracellular signaling pathways are involved in regulating the activity and expressions of K_v channels [10]. Finally, protein kinase modulation of potassium channels can regulate the gating kinetics, current amplitude, and influence the number of functional channels on plasmic membrane.

In the present study, we tested the possible action of lithium on I_K in hippocampal slices of rats and to determine the transduction pathways mediating the electrophysiological effects of lithium.

1 Materials and methods

1.1 Slice preparation

Male Wistar rats on postnatal days 14～18 were from Experimental Animal Center, Chinese Academy of Medical Sciences. The experiments were conducted in accordance with the guidelines of the Medical Experimental Animal Administrative Committee of the Nation. Horizontal slices that included the entire hippocampus and subiculum (400 μm in thickness) were prepared with a vibratome (VT1000M/E, Leica, Germany). Slices were maintained in artificial cerebrospinal fluid (ACSF) containing (in mmol/L): 125 NaCl, 25 $NaHCO_3$, 1.25 KCl, 1.25 KH_2PO_4, 1.5 $MgCl_2$, 2.0 $CaCl_2$, 16 Glucose. During the recordings, the slices were kept submerged in a chamber perfused with ACSF. In the experiments, the ACSF was saturated with 95% O_2/5% CO_2 and the temperature was kept at 30℃.

1.2 Electrophysiological recording

For whole-cell recording, CA1 neurons were visualized on a television monitor connected to a low light sensitive CCD camera (DVC710, USA). Whole-cell recording was performed using standard methods as described in detail previously [11]. Briefly, all experiments were conducted at room temperature (20～22℃). Micropatch pipettes (5～7 MΩ) were constructed from standard wall borosilicate glass on a two-stage puller.

Recordings were only made from cells where the seal resistance was greater than 1 GΩ. In whole-cell mode, cell membrane capacitance and series resistance were also electronically compensated. After seal formed and membrane ruptured, cells were allowed to stabilize for 3 min before starting formal pulse protocols.

Data acquisition and analysis were performed on computer using EPC10 patch-clamp amplifier (HEKA, Germany). Electrophysiological data were acquired and digitized at 20 kHz sampling rate and stored on a hard disk for off-line analysis.

1.3 Drug application

PDBu, U0126 were purchased from Alexis (USA) and ODQ (1H-[1, 2, 4] oxadiazole [4, 3-a] quinoxalin-1-one) was obtained from Cayman (USA). All drugs were prepared as stock solutions and diluted directly in ACSF to yield the appropriate concentration when used and the final DMSO (dissolved in dimethyl sulfoxide) concentration was < 1% respectively.

1.4 Data analysis

All data were analyzed by using Igor pro 5.04 and Origin 7.0. Peak current were obtained and conductance (G) was determined using the following formula $G = I/(V - V_k)$, where V_k is reversal potential. The peak conductance value for each test potential was normalized to G_{max} and plotted against the test potential to produce a voltage-conductance relationship curves, which were fitted using a Boltzmann equation: $G/G_{max}=1/\{1+\exp[-(V-V_h)/k]\}$, where G/G_{max} is the normalized conductance, V is the membrane potential, V_h is the voltage at which conductance being half-maximal, and k is the slope factor [12]. Data were presented as $\bar{x}\pm s$ and statistical analysis was performed by L.S.D comparison test. Statistical significance was considered at $P < 0.05$. All data analyses were perfor med using the software SPSS 13.0.

2 Results

To begin dissecting the roles of protein kinases in modulation of K^+ currents, we had to first isolate the

current from the myriad of currents activated by a voltage step in these cells. With Na^+ and Ca^{2+} currents eliminated pharmacologically, applying extra-cellular 10 μmol/L TTX and Cs^+ in the pipettes, relative noninactivating I_K currents were activated in response to a series of depolarizing pulses from a holding potential of −50 mV. This large transient current was almost completely blocked by application of 10 mmol/L TEA, which could partly recover after TEA was washed away (Figure 1).

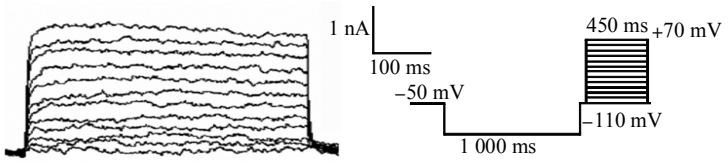

Fig. 1 Activation of I_K in CA1 cells by depolarizing voltage pulses

Currents generated by applying depolarizing pulses a 1 000 ms hyperpolarizing prepulse to −110 mV followed by interpulse in −50 mV for 50 ms and then step depolarized to potential from −50 to +70 mV for 450 ms with a 10 mV increment, the holding potential was −50 mV.

2.1 PKC decreases current of outwardly rectifying potassium channel (I_K)

As mentioned above, PKC has been implicated as a modulator of the potassium current in hippocampal dendrites and presented to be activated in memory lesion. Thus, we bath-applied the PKC activator PDBu (phorbol 12, 13-dibutyrate, 10 μmol/L) and measured the changes in the peak amplitude of current. It was found that 10 μmol/L PDBu was necessary to elicit the maximal inhibition effect on I_K, significantly decreasing the peak current. Typically, the inhibition was observed since 3 min after PDBu application, while it reached the maximum at 5 min. Relative current amplitudes of I_K clamped at 60 mV were (1.82±0.10) nA and (1.59±0.09) nA (n=6, P < 0.05) at 5 min after application of PDBu (Figure 2).

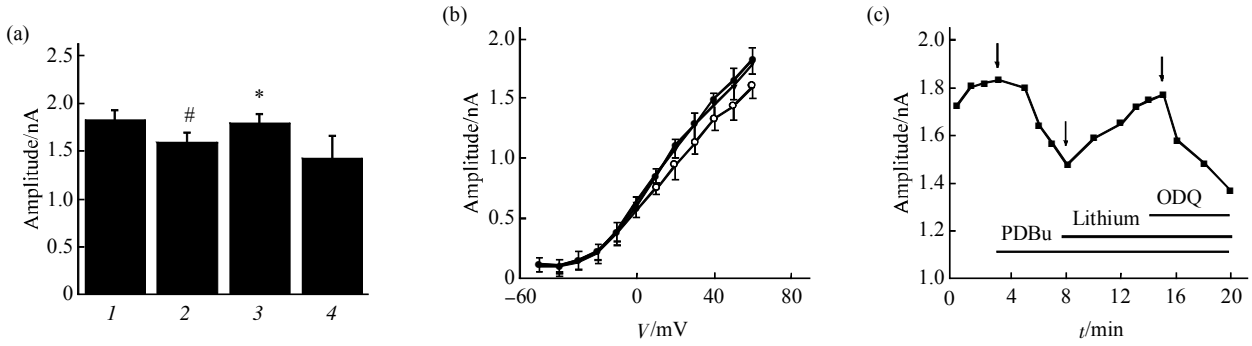

Fig. 2 Effects of protein kinase C (PKC), Lithium and ODQ on I_K (a, c)

Note that increase in I_K amplitude above PKC level following application of lithium carbonate (50 μmol/L), whereas after treatment of GC inhibitor ODQ (10 μmol/L), the current amplitude is suppressed much more. (b) shows the current-voltage relations for the peak currents. The maximal effect and relatively stable event were reached after the drugs were applied for 5 minutes. ●—●: Control; ○—○: PDBu; ▼—▼: Lithium. Data are the ($\bar{x}\pm s$) for all conditions tested. $^{\#}P$ < 0.05 PDBu group *vs* control group, $^{*}P$ < 0.05 PDBu+lithium group *vs* PDBu group, $F(2,15)$=11.149. *1*: Control; *2*: PDBu; *3*: PDBu+lithium; *4*: PDBu+lithium+ODQ.

To confirm that the effect of PDBu on I_K was a direct result of PKC activation, we tested the effects of the PKC inhibitor chelerythrine on potassium current. Bath-application of chelerythrine, the effect of PDBu on I_K was almost canceled, before application of PDBu, the amplitude was (1.47±0.17) nA, after treatment of PDBu, it reduced to (1.02±0.13) nA, with 10 μmol/L chelerythrine, the amplitude was reversed to (1.09±0.02) nA (P > 0.05), which likely as a result of nonspecific effects. In contrast to the effect of PDBu on I_K current, chelerythrine might enhance the activation of I_K channels, increasing the current.The steady-state activation curves of I_K channel in the presence and absence of PDBu were generated and fit

by a Boltzmann function (Figure 3), which suggested that after addition of PDBu, decreasing in $V_{1/2}$ (from (–1.68±2.64) mV to (–4.62±3.21) mV) and increasing in slopes (from (13.08±4.04) to (20.37±4.18)) were observed. These data reflected a decrease in maximum I_K steady-state amplitude at every voltage step in the activation protocol (n=6, P < 0.05). These data indicate that PDBu modulates delayed rectifier potassium channels, at least in part, by reducing both the total current produced by the channels and the percentage of channels available for activation.

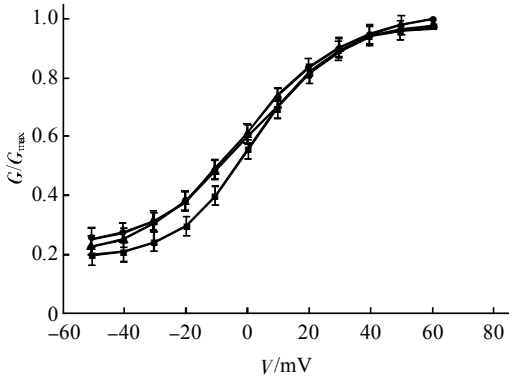

Fig. 3 Activation curves (Boltzmann functions) illustrating reduction in I_K by PKC activation and recovery in relative conductance after applying lithium

Each symbol represents the $\bar{x}\pm s$ current as relative conductance (G/G_{max}) elicited by a given depolarizing voltage step in the activation protocol calculated across CA1 cells. ■—■: Control; ●—●: PDBu; ▲—▲: PDBu + lithium.

2.2 Lithium carbonate reverses the inhibition effect of PDBu on I_K

Previous work has demonstrated that lithium treatments attenuated the stimulus-induced PKC translocations to a similar degree and decreased PKC activity in both cytosolic and membranous fractions (Manji *et al*., 2000). Therefore, it is notably to investigate the possibility whether lithium will reverse the inhibition effect of PKC activator on I_K. To determine whether lithium modulates potassium current in the context of PDBu, we examined the effect of lithium applying after the current reached the steady state. It is shown in Figure 2 that the presence of lithium carbonate (50 μmol/L) had reversing effects on PDBu-induced changes of I_K currents compared to only treatment with PDBu. The current amplitude changed from (1.59±0.09) nA in PDBu group to (1.79±0.09) nA after lithium was applied (n=6, P < 0.05). The steady-state activation curves of I_K channel in the presence of lithium after applying PDBu were fit by a Boltzmann function (Figure 3).

It was also found the midpoints to be shifted and significant difference between PDBu group and PDBu +lithium group, which changed from (–4.62 ± 3.21) mV to (–1.13±3.11) mV (n=6, P < 0.05) and slopes ranged from (20.37±4.18) to (17.32±4.48) (n=6, P > 0.05). When applying lithium directly on the brain slices without the effect of PDBu, no significant change could be observed, before application of lithium, the current amplitude was (1.09±0.33) nA, after 5 min of treatment of lithium it was (1.026 ± 0.340) nA (P > 0.05). These results indicate that inhibition effect of peak amplitude of potassium current by PDBu is attenuated after lithium application, and downstream signal activation might be involved in the changes.

2.3 ERK inhibitors block modulation effect of lithium on PKC inhibition of I_K

ERK/MAPK is important for regulation of neuronal function, particularly playing a role in the regulation of synaptic plasticity and long-term memory formation, which is through direct regulation of membrane ion channels, such as potassium channels. It has been demonstrated that lithium could control the pERK2 level[13]. The results discussed above show that the effect of lithium might be conducted by a downstream signal of PKC, acting to reverse the previous effect, which led us to question the pathway by which the kinases were acting. Both PKA and PKC activation can stimulate ERK[14].

To determine whether lithium blocked the PKC-induced suppression of the current depends on activation of the ERK pathway, we examined the effects of the MEK inhibitor U0126 on the PDBu-mediated decrease in potassium current. As shown in Figure 4, neurons were pre-exposed to 20 μmol/L U0126 for 20 min to inhibit activation of ERK[15]. When pretreated with U0126, although PDBu significantly reduced the current from (1.04±0.11) nA to (0.69±0.10) nA (n=6, P < 0.05), lithium did not reverse the effect of PDBu. After lithium was applied, the current changed to (0.72±0.08) nA (n=6, P > 0.05 *vs* PDBu group). These results are consistent with the hypothesis that PKC is acting upstream of ERK to modulate potassium channel, whereas blocking the MAPK pathway may abolish the neuroprotective effect

of lithium. It is therefore possible that changes in K^+ channel function mediated by the PKC-MAPK/ERK pathway could be one of underlying mechanisms related to neuroprotective effect of lithium.

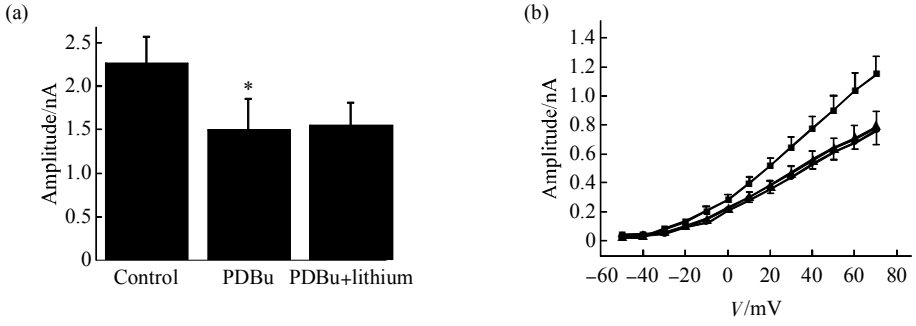

Fig. 4 Effects of protein kinase C (PKC), lithium and U0126 on I_K

Note that in (a), pretreatment with U0126 for 20 min, although PDBu significantly reduced the current, lithium did not reverse the effect of PDBu. (b) also shows the current change between different groups. ■—■: Control; ●—●: PDBu; ▲—▲: PDBu+lithium. Data are the $\bar{x}\pm s$ for all conditions tested, $^*P < 0.05$ *vs* control group.

2.4 AC-cAMP and GC-cGMP are involved in regulation of lithium on I_K

In the next series of experiments, we carried out a pilot study on further pharmacological mechanisms of lithium. When adenylate cyclase (AC) inhibitor MDL-12330A was performed, inhibit effect of lithium on presence of PDBu were nearly eliminated. As can be seen from the data, when pretreating with MDL-12330A(10 μmol/L), the peak current amplitude of control was (1.98 ±0.11) nA. After application of PDBu, it changed to (1.61 ±0.29) nA. With lithium, I_K was (1.22 ±0.37) nA (n =6, P < 0.05, Figure 5). The steady-state activation curves of I_K channel showed no significant change. These results elicited that under the condition that the PKC was activated, effects of lithium on PKC may be correlated with the activity of AC. As both PKA and PKC activation can stimulate ERK and other signal pathways, it is probable that inhibited AC resulted in dysfunction of MAPK/ERK signal transduction, which might show a further elimination of the effect of lithium. However, the exact machenism remains further investigation.

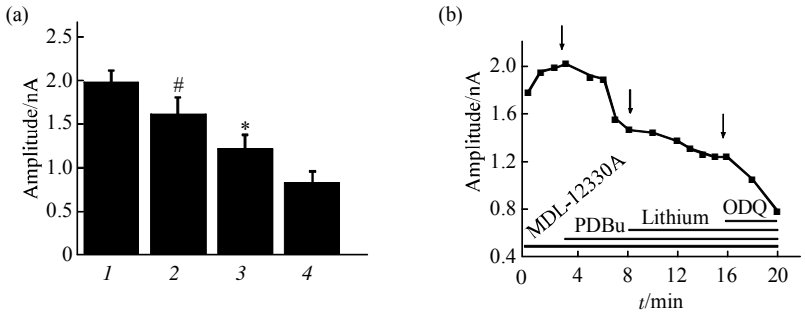

Fig. 5 Comparison of the effects of protein kinase C (PKC), lithium and ODQ under the condition of MDL-12330A′s pretreatment

The maximal effect and relatively stable event were reached after the drugs were applied for 5 min. Graph indicates I_K $\bar{x}\pm s$ for all conditions tested. The change of current amplitude is almost the same except for the lithium's treatment. Significant change can be seen between Figure 2 and Figure 5. $^{\#}P <$ 0.05 *vs* control group, $^*P < 0.05$ *vs* PDBu group, $F(2.15)=8.367$. *1*: Control; *2*: PDBu; *3*: PDBu+lithium; *4*: PDBu+lithium+ODQ.

To look for the downstream mechanism and with an attempt to further confirm the role of lithium in I_K regulation, the role of GC-cGMP pathway in I_K current was tested. In the present experiments, bath application of ODQ (1H-[1, 2, 4] oxadiazole [4, 3-a] quinoxalin-1-one)(10 μmol/L), which is an inhibitor of GC, led to a dramatic down-regulation of I_K (n =6) in both groups mentioned above (Figure 2, 5). However, when only ODQ was applied on the slices, it made no significant difference on I_K current, before application of ODQ, the current was (0.87 ±0.23) nA, after treatment of ODQ, it was (0.69±0.14) nA (P > 0.05).

The above findings possibly supported the idea that ODQ inhibited I_K under lithium's treatment.

3 Discussion

The roles of specific PKC isozymes in such cellular processes are still under investigation[16]. Studies on rats have implicated alterations in PKC activity as mediators of long-term alterations in neuronal excitability in the brain following acute stimulant use. High levels of PKC activity in the prefrontal cortex may contribute to a subset of symptoms involving the dysregulation of thought, affect, and behavior, which are features of many neuropsychiatric disorders[6]. However, the regulation of neuronal K^+ channels by PKC may be an important physiological mechanism for the modulation of membrane excitability and K^+ homeostasis [17]. These data suggest that direct activation of PKC through membrane-permeant activators inhibits K^+ channel currents. The K_V channel can be regulated by pharmacological activation of PKC, which decreases Kv current[18].

In most cases, protein kinases are thought to be "modulators" or modifiers of ion channel gating[19]. As in many tissues, K^+ channels are functional antagonists of voltage-dependent Ca^{2+} channels in the control of intracellular Ca^{2+} homeostasis. A shift in the activation curve is a change in the voltage dependence of the probability of channel opening, which indicates an effect on the activation gate of the channel[20].

Recently, it has been reported that voltage-dependent potassium channels, especially channels mediating I_K, might initiate apoptosis in some neurodegenerative disease[2]. In this study, under the condition of PKC activation, lithium can reverse its effect on delayed potassium channel. That shows lithium may exert neuroprotective effect through suppressing PKC pathway. The increase of I_K in neurons dramatically shortens the spike duration. Given that Ca^{2+} entry is primarily triggered by spikes in neurons, the shortened spike duration will certainly limit Ca^{2+} influx, and hence prevents the activation of cell death pathways that is due to the intracellular Ca^{2+} overload[21].

Currently available data suggest that acute lithium exposure facilitates a number of PKC-mediated responses, whereas exposure results in an attenuation of phorbol ester mediated responses, which is accompanied by a down-regulation of K^+ channels. In view of the pivotal role of the PKC signaling pathway in the regulation of neuronal excitability, neurotransmitter release, and long-term synaptic events, it was postulated that the attenuation of PKC activity may play a role in the effect of lithium[22].

Additionally, ERK has been shown to be involved in the regulation of A-type potassium channels in hippocampal CA1 pyramidal neurons[23], and inhibition and activation of this kinase modulates A currents[24]. ERK is known to phosphorylate Kv4.2, an A-type potassium channel subunit[25]. Because PKC, PKA, and ERK play important roles in modulation of potassium channels which may interact by means of phosphorylation, we sought to test in the context of lithium, whether these kinases modulate delayed potassium channels in brain sclices.

It is interesting to note that when pretreated with U0126, although PDBu significantly reduced the current, lithium did not reverse the effect of PDBu, which suggested that blocked MEK/ERK might interfer the effect of lithium on PKC activation. Therefore, blocking the MAPK pathway may abolish the neuroprotective effect of lithium, which suggests that MEK/ERK is required in the phosphorylation of potassium channel. Recent studies suggest that PKC might act on Raf, and PKA on B-Raf through Rap1 to activate ERK[26]. Thus one or more of above signals could be targets of lithium, resulting in the reverse effect on I_K without ERK activation. Future studies will endeavor to identify the specific regulator mediating the effect of lithium on potassium channel.

There is now also considerable evidence that chronic administration of "therapeutic" concentrations of lithium affects the function of second messenger generating systems in brain[5]. Masana *et al*.[27] demonstrated that chronic treatment of rats with lithium resulted in a significant increase in both basal and post-receptor stimulated AC activity. We further found that when pretreated with MDL-12330A, lithium did not reverse the effect of PDBu. It was reported that K_V channels are regulated by PKA, which was promoted by cAMP. The binding activity of PKA to cAMP, which reflects the functional integrity of the kinase, is profoundly altered in neurons after cerebral ischemia, raising a possibility that PKA might differentially regulate K_v channels before and after ischemia[28].

The cGMP-PKG pathway has been found to be important in the modulation of ion channels involved

in neuronal excitability[9]. What we have also shown in this study is that the cGMP-PKG pathway may a general inhibitory effect on lithium's effect. When pretreatment with AC inhibitor, the effect of lithium on PKC was abolished. However, long time recording may result in current running down. The precise effect of GC-cGMP pathway on lithium's action remains further investigation.

It is therefore possible that changes in potassium channel function mediated by the PKC-MAPK/ERK pathway described here could be one of the mechanism of therapy effect of lithium carbonate, which is associated with synaptic function. The molecular mechanisms for synaptic integration, synaptic plasticity, and learning are of great contemporary interest to neuroscientists, and the results from the present experiments suggest that potassium channel and the PKC signaling cascades along with MAPK/ERK pathways are important components of these phenomena, which could also be one of underlying mechanisms likely related to neuroprotective effect of lithium.

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摘要　碳酸锂可以用于治疗创伤和神经退行性疾病导致的脑部损伤．研究表明其保护效应与蛋白激酶 C(PKC)和胞外信号调节激酶(ERK)有关．研究表明 PKC 激动剂 PDBu 可以抑制延迟整流钾通道(I_K)电流并使其激活电压曲线向超极化方向移动．碳酸锂(50 μmol/L)可以抑制 PDBu 的反应．进一步的研究表明，预先加入 MEK/ERK 抑制剂 U0126(20 μmol/L)，碳酸锂不能逆转 PDBu 对 I_K 的作用．因此，PKC 和丝裂原活化蛋白激酶(MAPK)/ERK 级联反应通路可能在钾离子通道的磷酸化调节中起作用．另外，AC-cAMP 和 GC-cGMP 的交互作用也可能参与碳酸锂对 PKC 激活作用的调节，成为其神经保护作用的机制之一．

关键词　海马，碳酸锂，延迟整流钾通道，蛋白激酶 C，胞外信号调节激酶
学科分类号　Q42，Q51，Q74

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