行政院國家科學委員會補助專題研究計畫成果報告

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※細胞內訊息途徑對體積變化而引起的鉀離子　　　　※
※運輸的影響　　　　　　　　　　　　　　　　　　※
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計畫類別：V個別型計畫　　□整合型計畫

計畫編號：NSC 89－2320－B－040－025

執行期間：88 年 8 月 1 日至 90 年 7 月 31 日

計畫主持人：黃純健

共同主持人：

本成果報告包括以下應繳交之附件：

- □赴國外出差或研習心得報告一份
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- □出席國際學術會議心得報告及發表之論文各一份
- □國際合作研究計畫國外研究報告書一份

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一、中文摘要

在低張狀態下，多數哺乳類細胞先是體積漲大，而後恢復原來體積。此一細胞調節本身體積的功能，可能與鉀離子的運輸有關。在本實驗中，我們以血癌細胞為模型，研究其因體積變化而活化之鉀離子運輸途徑。我們發現，血癌細胞上因體積變化而活化的鉀離子運輸會受到 ouabain 及 bumetanide 的影響而降低，顯示此一鉀離子運輸可能與 Na^+/K^+ pump 及 $Na^+/K^+/Cl^-$ 共同運輸有關。由於此一因體積變化而活化的鉀離子運輸亦為 NPPB 所抑制，顯示此一因體積變化而活化的鉀離子運輸可能經由一特殊之陰離子通道(anion channel)。而在細胞內訊息(signaling)方面，此一因體積變化而活化的鉀離子運輸為 genistein 所抑制，因此，其似乎受到 tyrosine protein kinase 的調節，但可能並不受到 Gardos 通道的影響。

關鍵詞：鉀離子運輸、細胞體積、血癌細胞

Abstract

Under hypoosmotic condition, most mammalian cell types undergo regulatory volume decrease (RVD) and restore their original volume. In the current study, we characterize the K^+ transport in K562 leukemia cells under hypoosmolarity. The hypoosmotically-induced K^+ efflux was inhibited by ouabain and bumetanide, suggesting the involvement of Na^+/K^+ pump and $Na^+/K^+/Cl^-$ cotransport. Anion channel blocker NPPB significantly inhibited the hypoosmotically-induced K^+ efflux, suggesting the K^+ efflux may also via a specific anion channel. Moreover, this hypoosmotically-induced K^+ efflux was blocked by genistein, suggesting intracellular tyrosine protein kinase may regulate this hypoosmotically-induced K^+ efflux. Since the hypoosmotically -induced K^+ efflux was not blocked by EDTA, the involvement with Gardos channel may be unlikely.

Keywords: K^+ efflux, Cell volume, leukemia cells

緣由與目的

Under hypoosmotic condition, most mammalian cells first swell and then recover restore their original volume. This process has been termed RVD (regulatory volume decrease). The mechanisms of RVD are not clear, however, several pathways have been proposed to be involved with. Among them, K^+ transport was suggested to play a role on regulating RVD. In various cell types, K^+ and Cl^- channels, Cl^- dependent K^+ transporter, Gardos channel, Na^+/K^+ pump are suggested to be involved with the RVD regulatory mechanisms. In the current study, we try to characterize the RVD regulatory K^+ transport in K562 leukemia cells.

結果與討論

Under hypoosmotic condition, K562 cells increased their potassium efflux comparing to cells under isoosmotic condition. This hypoosmotically-induced K

efflux is significantly inhibited by ouabain and bumetanide, suggesting the involvement with Na^+/K^+ pump and $Na^+/K^+/Cl^-$ cotransporter. However, treating K562 cells with quinine did not result in significant inhibition of this hypoosmotically-induced K^+ efflux. Furthermore, treating cells with EDTA did not significantly affect the hypoosmotically-induced K^+ efflux. Thus, the involvement of Gardos channel with the hypoosmotically-induced K^+ efflux may be unlikely.

To further characterize this hypoosmotically-induced K^+ efflux, K562 cells were treated under Cl^- free conditions. Incubating with Cl^- free condition did not significantly alter this hypoosmotically -induced K^+ efflux. These results suggest that the hypoosmotically-induced K^+ efflux may not mediate by Cl^--dependent pathway. In some cell types, the hypoosmotically-induced K^+ efflux was suggested to be mediated by Cl^--dependent pathway. The lack of this Cl^--dependent pathway in K562 cells may worth to investigate further.

Since the hypoosmotically-induced K^+ efflux has been suggested to be possibly via a specific Cl^- channel, thus, we have characterized this K^+ efflux by using NPPB and genistein. Treating K562 cells with NPPB or genistein significantly inhibited the hypoosmotically-induced K^+ efflux in K562 cells. These results suggest the hypoosmotically-induced K^+ efflux in K562 cells may via a specific Cl^- channel and tyrosine protein kinase may anticipate with the regulatory processes of this hypoosmotically-induced K^+ efflux.

In conclusion, we have showed that K562 cells are capable of increasing K^+ efflux under hypoosmotic condition. This hypoosmotically-induced K^+ efflux is via $Na^+/K^+/Cl^-$ cotransport or/and a specific Cl^- channel but not Cl^- dependent pathway or Gardos channel.

計畫成果自評

本計劃之研究結果與計劃內容相符，並且達成預期目標。此一計劃之研究成果在學術上頗具價值，應已具備在學術期刊上發表的結果。主要的發現包括一些特殊與細胞體積調節之鉀離子運輸途徑，與先前其他實驗室在其他細胞之結果有相符與相異之處，頗值得再進一步探討。

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