行政院國家科學委員會專題研究計畫　成果報告

缺氧前置 訓練的機制-用蛋白質學方法探討

計畫類別： 個別型計畫
計畫編號： NSC92-2314-B-002-272-
執行期間： 92 年 08 月 01 日至 93 年 07 月 31 日
執行單位： 國立臺灣大學醫學院外科

計畫主持人： 賴逸儒
共同主持人： 張金堅

報告類型： 精簡報告

處理方式： 本計畫可公開查詢

中　華　民　國 93 年 11 月 2 日

行政院國家科學委員會補助專題研究計畫成果報告

※※※※※※※※※※※※※※※※※※※※※※※※※
※ ※
※缺氧前置訓練的機制-用蛋白質學方法探討※
※※※※※※※※※※※※※※※※※※※※※※※※※

計畫類別：V 個別型計畫　　□整合型計畫
計畫編號：NSC 92-2314-B-002-272
執行期間：92 年　8 月　1 日至 93 年 7 月 31 日

計畫主持人：賴逸儒
共同主持人：張金堅

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
- □赴大陸地區出差或研習心得報告一份
- □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

執行單位：臺大醫院外科部

中　華　民　國　93 年　10 月　29 日

行政院國家科學委員會專題研究計畫成果報告

國科會專題研究計畫成果報告撰寫格式說明

Preparation of NSC Project Reports

計畫編號：NSC 92-2314-B-002-272
執行期限：92 年 8 月 1 日至 93 年 7 月 31 日

主持人：賴逸儒　　臺大醫院外科部
共同主持人：張金堅　　臺大醫院外科部
計畫參與人員：陳朝峰　　臺大醫學院生理所

一、中文摘要

器官先經歷過短時間的缺血重灌流訓練後，再承受較長時間的缺血傷害時，器官耐受力大為增加。這個現象稱為缺血前置訓練(ischemic Preconditioning)。已知在模擬高山缺氧環境下飼養的動物，其心肌對缺血的耐受性較好。過去對於缺氧所誘發的前置訓練(Hypoxic Preconditioning) 的機制研究較少，本研究比較正常氧壓飼養的大鼠和低氧飼養2週的大鼠，藉蛋白質體學方法,以二維電泳和質譜儀分析其肝臟組織 探討前置缺氧訓練的機制。二維電泳和質譜儀分析的結果顯示低氧飼養2週的大鼠肝臟中，大量表現myosin 及 actin等和胞器骨架或訊息傳遞有關的蛋白質。吾人也以西方墨漬法分析確認myosin heavy chain (206DKa)的過度表現。至於myosin heavy chain的表現是否具有功能上的意義，則需進一步探討。

(Abstract)

Preconditioning is defined as a phenomenon in which an organ or tissue is rendered resistant to a more sustained insult by prior exposure to brief periods of injury. Altitude training, used by endurance athletes to improve sea-level performance, is an exercise under exposure of chronic hypoxia. Exposure of the rat to a period of hypoxia increases the cardiac tolerance to subsequent ischemic insults. The mechanism of hypoxic preconditioning remains to be elucidated. We use the proteomics approach to study thoroughly the important factors involved in the mechanism of hypoxic preconditioning, Wistar rats exposed to 2 weeks of hypoxia were used, the liver tissue of experiment animals are harvested as target organ analysis.

Two-dimensional gel electrophoresis was used to pick out the differentially-displayed protein spots for further analysis by mass spectrometry, and Protein database searching was performed. The proteins identified included myosin heavy chain, myosin polypeptide 9 (non-muscle type), myosin polypeptide 3, actin alpha1 and cytoplasmic beta-actin Western blot analysis confirmed the increased expression of myosin heavy chain (gi|8250661) in liver tissue of rats preconditioned by hypoxia.

Keywords: Hypoxia, Liver, myosin

二、緣由與目的

從事耐力運動的運動員，為了改善在平地的表現，所接受的高原訓練，類似在慢性缺氧的環境下的訓練運動。生物體對慢性缺氧環境的生理調適包括誘發紅血球生成素的製造和增加紅血球的量，結果會增加細胞攜帶氧氣的能力[1]。前人已從心臟前置訓練的研究中證實，將大鼠暴露在低氧環境中一段時間後，可以增加心臟承受接下來的缺血傷害，表示慢性缺氧可誘發細胞增強保護的能力[2]。關於低氧前置訓練的機制仍有待了解，只有少數研究提出和粒線體上的需能鉀離子通道（K_{ATP} channel）的功能有關[3]。

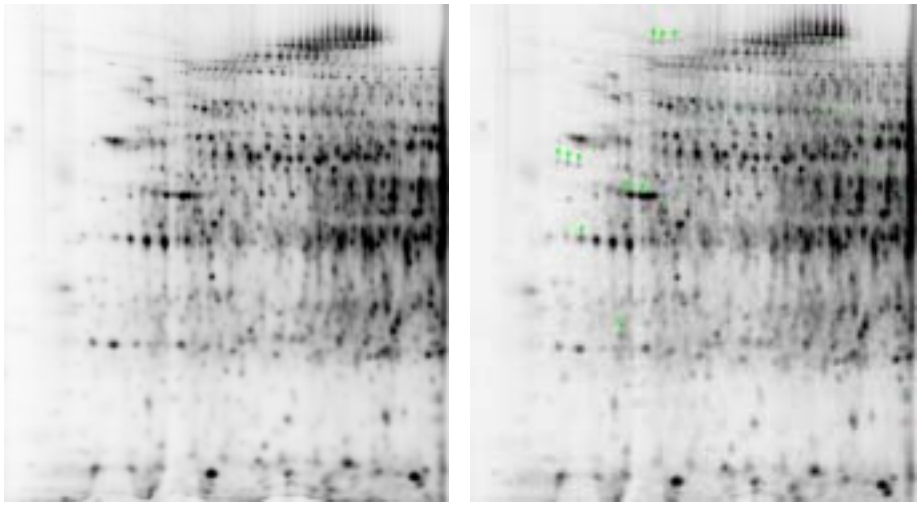
血基質氧化酵素是催化血基質成膽綠素和一氧化碳（carbon monoxide）的速率決定酵素[4]。前人研究發現，在細胞承受各種如低氧[5]（Poss, et al.1997）、熱休克[6]、自由基[7]等壓力時，會誘發出血基質氧化酵素的同分異構物，血基質氧化酵素-1。這種誘發反應被視為是具有保護作用的細胞反應[8]。吾人實驗已證實低氧前置訓練可誘發血基質氧化酵素-1的表現，並對於保護肝臟承受缺血再灌流的傷害[9]。

然而，解決生物學問題的方式已經從歸納還原（reductionist approach）進展到全面式的探討（global approach）。我們不再滿足於一次探討單一因子，而希望研究參與前置缺氧訓練的所有因子和其間的交互作用。關於前置缺氧訓練的機制研究較少且分散，本研究希望用蛋白質體學的方法，針對此問題作全面式的探討，期望能找出重要的關鍵因子，發展出具有前置訓練效果的藥物，應用到臨床上。

三、結果與討論

1．經過前置缺氧訓練2週 (hypoxia 2 weeks)和飼養於正常氧壓(control)的F344雄鼠，收集肝臟檢體，進行二維電泳分析，結果如圖1，綠色箭頭所指為缺氧訓練後肝臟中表現增加的蛋白質。

圖 1



Control　　Hypoxia 2weeks

2. 質譜儀分析和比對 EST 序列資料庫，得到 5 種表現增加的蛋白質的身份如下

(1) gi|8250661 myosin heavy chain [Rattus sp.]

(2)**gi|6981236| myosin, heavy polypeptide 9, non-muscle** [Rattus norvegicus] gi|13431671|sp|Q62812|MYH9_RAT Myosin heavy chain, nonmuscle type A (Cellular myosin heavy chain, type A) (Nonmuscle myosin heavy chain-A) (NMMHC-A) gi|967249|gb|AAA74950.1| nonmuscle myosin heavy chain-A

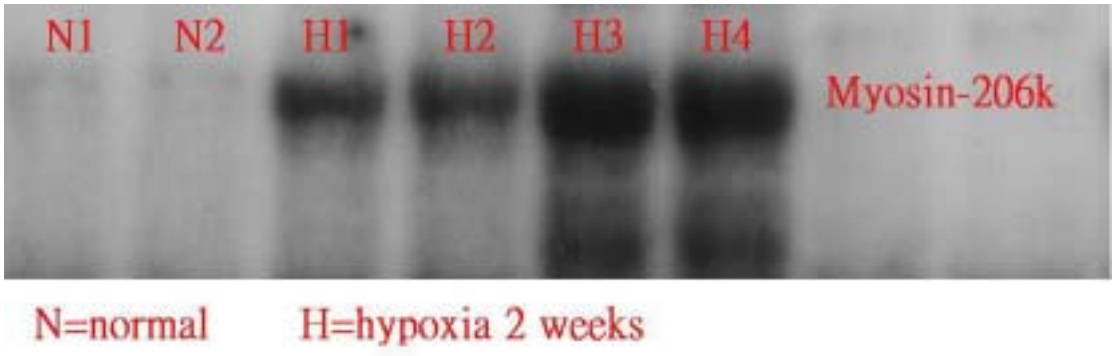
(3)**gi|6981234| myosin, heavy polypeptide 3, skeletal muscle, embryonic** [Rattus norvegicus] gi|127755|sp|P12847|MYH3_RAT Myosin heavy chain, fast skeletal muscle, embryonic gi|92509|pir||A24922 myosin heavy chain, skeletal muscle, embryonic - rat gi|1619328|emb|CAA27817.1| myosin heavy chain [Rattus norvegicus]

(4) **gi|9506371|ref|NP_062085.1| actin alpha 1; actin, alpha 1, skeletal muscle** [Rattus norvegicus]gi|4501881 39 spectra zTot: 0.0e0 >gi|4501881|ref|NP_001091.1| alpha 1 actin precursor; alpha skeletal muscle actin [Homo sapiens] gi|11425324|ref|XP_001869.1| similar to Chain B, The X-Ray Crystal Structure Of The Complex Between Rabbit Skeletal Muscle Actin And Latrunculin A At 2.85 A Resolution [Homo sapiens] gi|20887491|ref|XP_134551.1| similar to actin, alpha 1, skeletal muscle [Rattus

norvegicus] [Mus musculus] gi|113287|sp|P02568|ACTS_HUMAN Actin, alpha skeletal muscle (Alpha-actin 1) gi|71610|pir||ATHU actin alpha 1, skeletal muscle gi|90264|pir||A24904 actin alpha, skeletal muscle - mouse gi|7441425|pir||JC5301 skeletal alpha-actin - pig gi|20663851|pdb|1IJJ|B Chain B, The X-Ray Crystal Structure Of The Complex Between Rabbit Skeletal Muscle Actin And Latrunculin A At 2.85 A Resolution gi|20663850|pdb|1IJJ|A Chain A, The X-Ray Crystal Structure Of The Complex Between Rabbit Skeletal Muscle Actin And Latrunculin A At 2.85 A Resolution gi|55577|emb|CAA24529.1| actin [Rattus norvegicus] gi|63029|emb|CAA24753.1| a-actin [Gallus gallus] gi|178029|gb|AAB59376.1| alpha-actin gi|309088|gb|AAA37164.1| actin gi|337746|gb|AAA60296.1| alpha-skeletal actin precursor gi|387081|gb|AAA37141.1| alpha-actin gi|790202|gb|AAC48692.1| skeletal alpha actin gi|6049633|gb|AAF02694.1|AF182035_1 skeletal muscle alpha-actin precursor [Homo sapiens] gi|15214923|gb|AAH12597.1|AAH12597 Similar to actin, alpha 1, skeletal muscle [Homo sapiens] gi|15928834|gb|AAH14877.1| actin, alpha 1, skeletal muscle [Mus musculus] gi|223503|prf||0809315A actin

(5) gi|13592133| cytoplasmic beta-actin [Rattus norvegicus]gi|4501885 8 spectra zTot: 0.0e0 >gi|4501885|ref|NP_001092.1| beta actin; beta cytoskeletal actin [Homo sapiens] gi|6671509|ref|NP_031419.1| actin, beta, cytoplasmic; A-X actin-like protein; melanoma X-actin [Mus musculus] gi|113270|sp|P02570|ACTB_HUMAN Actin, cytoplasmic 1 (Beta-actin) gi|71618|pir||ATHUB actin beta - human gi|71619|pir||ATMSB actin beta - mouse gi|279669|pir||ATCHB actin beta - chicken gi|28252|emb|CAA25099.1| beta-actin [Homo sapiens] gi|49866|emb|CAA27307.1| beta-actin (aa 1-375) [Mus musculus] gi|55575|emb|CAA24528.1| beta-actin [Rattus norvegicus] gi|177968|gb|AAA51567.1| cytoplasmic beta actin gi|211237|gb|AAA48615.1| beta-actin gi|2116655|dbj|BAA20266.1| beta-actin [Cercopithecus aethiops] gi|2182269|gb|AAB60717.1| beta actin gi|2661136|gb|AAB88212.1| beta actin [Equus caballus] gi|3320892|gb|AAC26519.1| beta-actin [Trichosurus vulpecula] gi|12654911|gb|AAH01301.1|AAH01301 actin, beta [Homo sapiens] gi|12803203|gb|AAH02409.1|AAH02409 actin, beta [Homo sapiens] gi|13279023|gb|AAH04251.1|AAH04251 actin, beta [Homo sapiens] gi|14041683|emb|CAC38394.1| beta actin [Mesocricetus auratus] gi|14424511|gb|AAH09275.1|AAH09275 actin, beta [Homo sapiens] gi|15426536|gb|AAH13380.1|AAH13380 actin, beta [Homo sapiens] gi|15928803|gb|AAH14861.1|AAH14861 actin, beta [Homo sapiens] gi|21070355|gb|AAM34270.1|AF508792_1 beta actin [Cavia porcellus] MDDDIAALVV

3. 西方墨點分析(Western blotting analysis)驗證質譜儀的分析結果，得到 Myosin heavy chainy 在肝臟組織的表現增加情形:



四、計畫成果自評

本實驗初步驗證 hypoxic precoditioning 會增加 myosin heavy chain 在肝臟的表現，推測可能改變肝臟的細胞結構(cytoskeleton) 、重塑(remodeling) 或者和訊息傳遞(signal transduction)有關。相關結果迄今僅有篇論文提及 [10] 應該有發表的價值。至於這種細胞結構的重塑是永久或暫時的、以及這種重塑是否有功能上的意義，例如能否增加肝臟對缺氧的耐受性，則有待進一步研究。本實驗因受限於經費及時間，無法對其他二維電泳分析所找出的蛋白質做分析， 較為可惜。

五、參考文獻

1. Levine BD. Stray-Gundersen J. "Living high-training low": effect of moderate-altitude acclimatization with low-altitude training on performance. *J. Appl. Physiol.* 1997;83:102-12
2. Asemu G. Papousek F. Ostadal B. Kolar F. Adaptation to high altitude hypoxia protects the rat heart against ischemia-induced arrhythmias. Involvement of mitochondrial K_{ATP} channel. J Mol Cell Cardiol. 1999; 31:1821
3. Ren Z, Yang Q, Floten HS, He GW. Hypoxic preconditioning in coronary microarteries: role of EDHF and K+ channel openers. Ann Thorac Surg 2002 74:143
4. Augustine M, Choi K, Alam J. Heme oxygenase-1: Function, regulation, and implication of a novel stress-inducible protein in oxidant-induced lung injury. *Am.* J Respir Cell Mol Biol 1996;15: 9

5. Poss KD, Tonegawa S. Reduced stress defense in heme oxygenase 1-deficient cells. *Proc.Nat. Acad.Sci*. U.S.A. 1997;94: 10925-30
6. Lee PJ, Jiang BH, Chin BY et al. Hypoxia-inducible factor-1 mediatess transcriptional activiation of the heme oxygenase-1 gene in response to hypoxia. *J.Bio.Chem*. 1997;272:5375-81
7. Gabis KK, Gildemeister OS, Pepe JA, Lambrecht RW, Bonkovsky HL. Induction of heme oxygenase-1 in LMH cells. Comparison of LMH cells to primary chicken embryo liver cells. Biochim Biophys Acta 1996;1290:113
8. Lu TH, Lambrecht RW, Pepe J, Shan, Y.; Kim, T.; Bonkovsky, H.L. Molecular cloning, characterization and expression of the chicken heme oxygenase-1 gene in transfected primary cultures of chicken embryo liver cells. Gene 1998;207:177
9. Lai IR, Ma MC, Chen CF, Chang KJ. The protective role of heme oxgenase-1 on liver after hypoxic preconditioning in rats. *Tranplantation* 2004;77:1004
10. Ogata I, Saez CG, Greenwel P, Ponce Mde L, Geerts A, Leinwand LA, Rojkind M. Rat liver fat-storing cell lines express sarcomeric myosin heavy chain mRNA and protein. Cell Motil Cytoskeleton. 1993;26:125-32.