

# 嘉南藥理科技大學專題研究計畫成果報告

計畫編號：CN9924

計畫名稱：探討介白質 10 之解熱作用是否經由抑制兔子下視丘麩胺酸  
-氫氧自由基-前列腺素 E<sub>2</sub> 途徑所致

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# 摘要

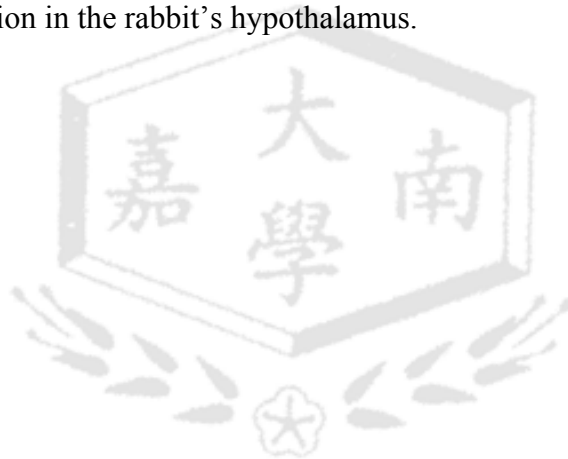
最近的研究顯示介白質-10(interleukin-10, IL-10)可作為內生性解熱原，並可透過抑制發炎性細胞激素如介白質-1 $\beta$ ((interleukin-1 $\beta$ , IL-1 $\beta$ ), 介白質-6 (IL-6) 及腫瘤壞死因子(tumor necrosis factor- $\alpha$ , TNF- $\alpha$ )而來調控脂多醣(lipopolysaccharide, LPS)所引起之發燒反應。除了致熱性細胞激素(pyrogenic cytokines)外，證據顯示其他內生性物質，特別是麩胺酸(glutamate)、氫氧自由基(hydroxyl radicals)、前列腺素E<sub>2</sub> (prostaglandin E<sub>2</sub>, PGE<sub>2</sub>)都被認為之發燒有關。就我們所知，目前對於IL-10減少LPS之發燒反應是否經由抑制兔子下視丘麩胺酸-氫氧自由基-前列腺素E<sub>2</sub>之途徑，仍然所知有限。因此，本研究將探討IL-10是否經由抑制靜脈注射LPS誘導下視丘麩胺酸、氫氧自由基、前列腺素 E<sub>2</sub>之產生，而達到解熱作用。主要是利用先前埋於兔子下視丘前視區(preoptic region of anterior hypothalamus, POAH)之微透析探針來收集細胞外之透析物。氫氧自由基之測量則是利用水楊酸鹽(salicylate)可和氫氧自由基形成穩定的2, 3-二羥基苯甲酸(2, 3-dihydroxybenzoic acid, 2, 3-DHBA)之特性，來測量下視丘中2, 3- DHBA，以代表氫氧自由基之量，而麩胺酸則利用高效液體層析法(high performance liquid chromatography, HPLC)結合螢光偵測器來測量；前列腺素E<sub>2</sub>之濃度是以酵素連接免疫吸附法(enzyme-linked immunosorbent assay, ELISA)來測量。而且，我們將進一步利用electrophoretic mobility shift assay(EMSA)及西方墨點法探討IL-10減少下視丘前列腺素E<sub>2</sub>之產生是否經由抑制NF- $\kappa$ B活化及向下調控下游環氧化酶-2 (cyclooxygenase-2, COX-2)蛋白表現所致。因此，本研究計畫結果將可揭露IL-10可能透過抑制LPS在兔子下視丘誘導產生之麩胺酸、氫氧自由基及前列腺

E<sub>2</sub>，而達到解熱作用。

## ABSTRACT

Recent reports have shown that interleukin-10 (IL-10) functions as an antipyretic in the regulation of fever to lipopolysaccharide (LPS) by inhibiting the synthesis of proinflammatory cytokines, including interleukin 1  $\beta$  (IL-1  $\beta$ ), IL-6 and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ). Besides pyrogenic cytokines, evidence has accumulated to suggest that other endogenous compound particular glutamate, hydroxyl radicals, and PGE<sub>2</sub> have been considered to be involved in the genesis of pyrogenic fever. To our knowledge, little is known about that the LPS-induced fever could be reduced by IL-10 via inhibiting of glutamate-hydroxyl radical-prostaglandinE<sub>2</sub> (PGE<sub>2</sub>) pathway in the rabbit's hypothalamus. Therefore, the present study will attempt to determine whether IL-10 exerts its antipyresis by suppressing overproduction hypothalamic concentration of glutamate, hydroxyl radicals, and PGE<sub>2</sub> induced by peripheral administration of LPS in rabbits. The extracellular dialysates in the preoptic region of the anterior hypothalamus (POAH) will be collected using a microdialysis probe previously placed in the brain region. The salicylate trapping method will be used to detect hydroxyl radicals by measurement of the stable product 2,3-dihydroxybenzoic acid (2,3-DHBA), whereas the glutamate release in the anterior hypothalamus of rabbit brain will be measured by high performance liquid chromatography (HPLC) in combination with a

fluorescence detector. The concentration of PGE<sub>2</sub> in the extracellular dialysate will be detected by enzyme-linked immunosorbent assay (ELISA) kits. In addition, we will use electrophoretic mobility shift assay (EMSA) as well as western blot to determine whether IL-10 reduces LPS-induced hypothalamic level of PGE<sub>2</sub> may act through inhibiting NF-κB activation and down regulation of cyclooxygenase-2 (COX-2) protein expression in the anterior hypothalamic tissue. The results obtained from this proposal should unveil that IL-10 may exert its amelioration of LPS-induced fever by inhibiting the glutamate, hydroxyl radical and PGE<sub>2</sub> production in the rabbit's hypothalamus.



# 報告內容

## (一) 研究目的：

生物體溫恆定之維持，主要是受到下視丘前視區(preoptic region of the anterior hypothalamus, POAH)的調控，因其內含有許多對溫度變化敏感的神經元，可以有效地整合來自中樞或週邊的體溫接受器(thermoreceptors)所傳入的訊息，感應外界環境溫度的變化，而使體溫維持在一定的溫度，即體溫設定點(set-point temperature)(Boulant, 1998)。發燒是臨床疾病上常被描述的症狀，尤其常見於因微生物感染所引起的發炎反應，其定義為下視丘體溫調控中樞之設定點(set point of thermoregulatory center)高於原來的設定點，使得體溫高於正常之恆定溫度(Kluger, 1991)。目前有關於熱原性發燒之研究，以細菌內毒素(endotoxin)或脂多糖(lipopolysaccharide, LPS)所引起發燒反應之研究最為詳盡。LPS是革蘭氏陰性菌(Gram negative bacteria)細胞壁外膜的主要成份，細菌內毒素進入體內能引起各式各樣的臨床症狀，如畏寒(chills)、發燒、頭痛(headache)、敗血症(sepsis)、敗血性休克(septic shock)(Parrillo, 1993)。在美國，每年大約有21萬人死於敗血症(Angus, et al., 2001)，而且敗血性休克的死亡率，更高達50%(Salvo et al., 1995)。目前LPS引起發燒的機轉，可能是LPS刺激單核球(monocyte)或巨噬細胞(macrophage)產生一些致熱性細胞激素(pyrogenic cytokines)如TNF- $\alpha$ 、IL-1、IL-6，而來導致發燒(Luheshi, 1998; Dinarello, 1999)。至於這些血液中的pyrogenic cytokines如何作用在腦部，而引起發燒反應。目前的可能機轉是這些pyrogenic cytokines隨著血液循環進入位於第三腦室前面，視交叉(optic chiasma)上面的一個circumventricular organ，即所謂血管脈叢終端器官(organum vasculosum laminae terminalis, OVLT)，由於OVLT靠近下視丘體溫調控中樞，而且此區域含有豐富微血管及缺乏血腦障礙(blood brain barrier)(Hashimoto et al., 1994)，所以可允許一些血液中之致熱原進入此區域，特別是IL-1，而刺激OVLT附近的內皮細胞、微小膠細胞中cyclooxygenase (COX-2)基因之表現(Lacroix and Rivest, 1998; Quan et al., 1998)，進而產生前列腺素E<sub>2</sub>(prostaglandin E<sub>2</sub>, PGE<sub>2</sub>)(Komaki et al., 1992)，這些PGE<sub>2</sub>可直接擴散至下視丘前視區(Stitt, 1986)或作用分佈在OVLT內，而投射至下視丘的神經元(Ota et al., 1997)，進而提昇體溫控制中樞之設定點，使身體增加產熱、減少散熱，進而導致發燒的現象(Saper and Breder, 1994)。目前所知除了PGE<sub>2</sub>是致熱源引起發燒的重要媒介物質外，最近研究亦發現glutamate和熱原性發燒反應有關。利用SEA靜脈注射至兔子內，並利用微透析(microdialysis)方法在OVLT區域收集腦脊髓液，結果發現SEA在兔子內可引起發燒，並造成OVLT區域glutamate之上升，而且SEA所引起之發燒與glutamate之釋放都可被NMDA receptor antagonist MK-801及ketamine所抑制(Huang et al., 2001)；除此之外，我們亦發現臨床常用的解熱劑acetaminophen及aspirin都可透過抑制glutamate的釋放，而來達到解熱的作用(Huang et al., 2003a; Huang et al., 2003b)。

最近的研究亦發現麩胺酸(glutamate)及氫氧自由基(hydroxyl radical)和LPS的發燒有關(Huang et al., 2006; Kao et al., 2007a)。Abramov等人發現在cortical neurons因oxygen and glucose deprivation (OGD)造成glutamate的釋放，可透過增加細胞內Ca<sup>2+</sup>的濃度，而活化NADPH oxidase，而造成ROS的增加(Abramov et al., 2007)，而且在老鼠腦部紋狀體(striatum)給予glutamate，利用微透析技術都可測得hydroxyl radical有

增加的現象(Lancelot et al., 1998a; Lancelot et al., 1998b)。同樣地，在體外(in vitro)實驗當中，亦發現NMDA 接受器之活化可造成細胞產生大量的oxygen-derived free radical (Lafon-Cazal et al., 1993; Lafon-Cazal et al., 1995; Culcasi et al., 1994; Gunasekar et al., 1995)。可見，LPS可能是透過glutamate之釋放，進而誘導hydroxyl radical之產生，而引起發燒反應。

## ROS and Fever

所謂反應性氧自由基 (reactive oxygen species; ROS) 是泛指原子之電子軌道有不成對電子且具有高度反應性，可氧化或還原其他原子，其包含許多種類的自由基，例如：氫氧自由基 (hydroxyl radical; OH<sup>·</sup>)、超氧自由基 (superoxide radical; O<sub>2</sub><sup>·-</sup>)、過氧化氫 (hydrogen peroxide; H<sub>2</sub>O<sub>2</sub>)、一氧化氮自由基 (nitric oxide; NO<sup>·</sup>) 等化合物 (Bal-Price et al., 2002; Ciuseppe, 2002; Sies, 1993)，這些自由基可由體內許多細胞產生，擔任重要生理功能。其中過氧化氫 (H<sub>2</sub>O<sub>2</sub>) 是一個很特別的分子，可自由通過細胞膜，且在金屬離子催化下 Fenton reaction, Haber Weiss reaction) 自行分解為氫氧自由基 (hydroxyl radical; OH<sup>·</sup>)，氫氧自由基是一種反應性超強的自由基，在正常生理狀態下，細胞本身所釋出的自由基具有防禦的功能，然而在異常大量產生情況下，由於其含有不成對電子，因此非常不穩定，經常會攻擊細胞內之大分子，如脂質、去氧核糖核酸、蛋白質之共價鍵，而引起脂質氧化、DNA受損及破壞電子傳遞鏈的酵素活性，因此造成細胞能量衰竭而死亡 (Cuzzocrea et al., 2001)。除此之外，自由基亦和腦部缺血、中風所造成的神經傷害；內毒素所引起之休克、發炎反應、及動脈硬化 (atherosclerosis) 有關 (Gunasekar et al., 1995; Guo and Ward, 2000; Podrez et al., 2000; Yang and Lin, 2002)。最近的研究亦發現 reactive oxygen species 和熱原性之發燒有關。Riedel 等人將 LPS 週邊給予大白鼠或兔子可在血漿中測得 oxygen radical 有增加現象，而且利用一些 hydroxyl radical scavenger 如 methylene blue、 $\alpha$ -lipoic acid 及 aspirin 都可抑制 LPS 所引起之發燒及血漿 oxygen radical 之產生 (Riedel and Maulik, 1999; Riedel et al., 2003)。最近研究結果亦證實靜脈注射 LPS 可造成腦部 hydroxyl radical 的增加，而且和 LPS 的劑量呈現正相關，利用抗氧化劑  $\alpha$ -lipoic acid 及 aspirin 都可抑制 LPS 所誘導腦部 hydroxyl radical 的產生，而達到解熱的效果 (Huang et al., 2006; Kao et al., 2007b)。同樣地，我們發現中藥黃芩苷 (baicalin) 的解熱作用亦是透過抑制 hydroxyl radical 的產生所致 (Tasi et al., 2006)，由此可知在 LPS 發燒過程中確實會有 hydroxyl radical 之參與。目前許多證據顯示 LPS 可刺激腦部膠細胞產生 ROS，而活化 NF- $\kappa$ B (Dimayuga et al., 2007; Lee et al., 2006)。最近，我們的研究發現 NF- $\kappa$ B 亦和熱原性發燒有關，利用 NF- $\kappa$ B 抑制劑如 pyrrolidine dithio carbamate (PDTC) sodium pyrrithione 及 N-acetyl-L-cysteine (NAC) 靜脈注射至兔子內都可抑制 LPS (Lee et al., 2003) 及 SEA (Shao et al., 2004) 刺激週邊血單核細胞 (peripheral blood mononuclear cells, PBMC) 所產生之 IL-1 $\beta$ 、TNF- $\alpha$  及 IL-6，進而抑制其所引起之發燒反應。Kozak 等人將 LPS 靜脈注射至 NF- $\kappa$ B 基因缺乏的小鼠中，發現並不會引起發燒反應 (Kozak et al., 2006)，由此可知週邊 NF- $\kappa$ B 的活化在熱原性發燒扮演重要角色。先前研究顯示靜脈注射 LPS, IL-1 $\beta$ , TNF- $\alpha$  都可在老鼠腦部內皮細胞及小神經膠細胞 (microglia) 偵測到 COX-2 基因之表現 (Lacroix and Rivest, 1998; Quan et al., 1998)。事實上，先前的研究已證實 LPS，金黃色葡萄球菌腸毒素 A (staphylococcal

enterotoxin A, SEA) 及IL-1 $\beta$ 都可以透過活化腦部iNOS-COX-2途徑，而在兔子內引起發燒反應 (Huang et al., 1997; Lin and Lin, 1996)，然而對於外生性致熱原LPS所引起之發燒是否透過腦中產生之ROS而活化NF- $\kappa$ B，並導致COX-2大量表現及PGE<sub>2</sub>的產生，而引起發燒反應，目前仍然未知。

## IL-10 and fever

介白質-10(interleukin-10, IL-10)是一種由Th2淋巴球所產生之抗發炎性細胞激素 (Fiorentino, et al., 1989)，其可抑制發炎性細胞激素如TNF- $\alpha$ , IL-1, IL-6之產生(Howard & O'Garra, 1992; Jenkins et al., 1994)。IL-10因可降低TNF- $\alpha$ 的釋放而能保護老鼠免死於致死性內毒血症 (lethal endotoxaemia) (Gerard et al., 1993; Marchant et al., 1994)；若利用抗體中和內生性IL-10，將會增加發炎性細胞激素之產生，而促進內毒血症之死亡率(Standiford et al., 1995)。IL-10因可抑制發炎性細胞激素之產生，研究發現其亦具有抗發燒之特性。皮下注射IL-10，可抑制LPS所引起之發燒及IL-6之產生；相反地若用抗體中和內生性IL-10，則可增加LPS所誘導之發燒反應及IL-1 $\beta$ 、TNF- $\alpha$ 及IL-6之產生(Cartmell et al., 2003)。同樣地，在IL-10基因剔除(knockout)的小鼠，其對腹腔注射LPS所引起之發燒反應及血漿中IL-6的含量都較正常老鼠為高(Leon, et al., 1999)，而且腦室注射IL-10，亦可抑制腦室注射LPS所引起之發燒及在腦內下視丘及腦幹所產生之IL-1 $\beta$ (Ledeboer et al., 2002)。由此可見，IL-10確實可作為內生性解熱原(antipyretic)。最近的研究發現利用adenoviral vector將人類IL-10基因打入大白鼠之側腦室，發現可抑制因心肌梗塞而活化NF- $\kappa$ B之活性，並減少其下游產物COX-2在下視丘的表現及腦脊髓液PGE<sub>2</sub>的產生，同時也抑制下視丘IL-1 $\beta$ mRNA之表現(Yu et al., 2007)。同樣地，腹腔注射IL-10可減少腹腔注射LPS所造成NF- $\kappa$ B之活化，而抑制COX-2蛋白表現，進而降低內毒血症之死亡率(Ejima et al., 2003)。最近本人的研究發現腦室注射IL-1 $\beta$ 可增加腦脊髓液中麩胺酸及氫氧自由基之含量，而且腦室注射IL-1接受器拮抗劑(IL-1receptor antagonist)可抑制靜脈注射LPS所引起之發燒及腦中麩胺酸、氫氧自由基及PGE<sub>2</sub>(Huang, et al., 2008, unpublished data)之含量。因此，本計畫將探討IL-10是否經由抑制LPS所誘導麩胺酸、氫氧自由基及之PGE<sub>2</sub>產生，而達到解熱作用。

## The specific aim of present study

先前的研究發現LPS(Lee et al., 2003)及SEA (Huang et al., 1997; Won et al., 2000)都可刺激週邊血單核細胞產生IL-1 $\beta$ 、TNF- $\alpha$ 及IL-6而引起發燒反應，而且不論是週邊給予IL-10(Cartmell et al., 2003)或腦室給予(Ledeboer et al., 2002)都可因抑制LPS所刺激產生之IL-6或IL-1 $\beta$ ，而抑制LPS之發燒反應。最近我們研究成果證實，LPS發燒機轉和腦部glutamate及hydroxyl radical的增加有密切關係 (Huang et al., 2006)。然而對於IL-10是否經由抑制LPS所誘導腦中glutamate及hydroxyl radical之產生，而達到解熱作用，目前仍然未知。因此，本計畫將觀察腦室注射IL-10對於靜脈注射LPS所引起之發燒反應及刺激兔子腦部產生glutamate及hydroxyl radical及之影響。另外，因IL-10可透過調控NF- $\kappa$ B之活性，而抑制一些發炎物質之產生 (Al-Ashy, et al., 2006; Inoue et al., 2004)，因此我們將進一步探討IL-10是否可透過抑制LPS刺激腦部NF- $\kappa$ B之活化，而減少COX-2之表現及PGE<sub>2</sub>之產生，進而抑制LPS發燒反應。我們將利用腦室注射IL-10，來觀察其對靜脈注射LPS所引起之發燒反應及NF- $\kappa$ B活化、COX-2表現及PGE<sub>2</sub>產生之影響。

總之，本計畫之執行若能得到預期的結果，則對於IL-10之解熱機轉將有更深入之了解，除了透過抑制致熱性細胞激素之產生外，可能也透過抑制腦部glutamate及hydroxyl radical之產生，而影響下游NF- $\kappa$ B之活化、COX-2表現及PGE<sub>2</sub>之產生，而來達到解熱作用

## (二) 研究方法：

### (1) Surgical Techniques

Adult male New Zealand White rabbits, weighing 2.0-3.2 kg at the start of the study, will be used. Animal chow and tap water will be available ad libitum. The ambient temperature ( $T_a$ ) in the animal room was  $22 \pm 1^\circ\text{C}$ ; light and darkness will be alternated, with light on from 6:00 a.m. to 6:00 p.m.

The animals will be received a prophylactic injection of the antibiotic, gentamicin sulfate (18 mg/kg, im), and 1 h later will be anesthetized with sodium pentobarbital (30 mg/kg, iv). A sterile, microdialysis probe guide cannula with an indwelling stylet will be implanted stereotaxically into the left preoptic anterior hypothalamus (POAH) of each rabbit [coordinates, in mm, A2.5, L2.0, V15] or 6-mm-long, 17-gauge, thin-wall stainless steel into the right lateral cerebral ventricle [coordinates, in mm, P 4.0, R 3.0, V5] according to the atlas of Sawyer et al. (1954) and fixed to the skull with dental acrylic cement and four self-tapping, miniature, stainless steel screws. After surgery, the guide cannula will be plugged with a stylet, and animals will be returned to their cages for a minimal recovery period of 1 week.

### (2) Animals and Pyrogen Assay

Experiments will be conducted between 0900 and 1900 h, with each animal being used at an interval of not less than 7 days. Throughout the experiment, colonic temperatures will be measured every minute with a copper constantan thermocouple connected to a thermometer (HR1300, Yokogawa, Tokyo, Japan). The colonic temperature of each animal will be allowed to stabilize for at least 120 min before any injections. Only animals whose body temperatures are stable and in the range of  $38.6$  to  $39.7^\circ\text{C}$  will be used to determine the effect of drug application. All experimental animals will be obtained from the animal center of Chi-Mei Medical Center. (Tainan, Taiwan, ROC). The animal protocol described here will be approved by the animal ethical committee of Ch-Mei Medical Center.

### (3) Microdialysis for detection extracellular glutamate

At least 1 h before an experiment, the indwelling stylet of the guide cannula will be replaced by a CMA-12 microdialysis probe purchased from CMA/Microdialysis (RosLagsvågen, Stockholm, Sweden) so that its dialysis membrane tip protruded exactly 4.0 mm beyond the guide tube. Dialysate samples will be collected at 20-min intervals over a period of 10 hours, and stored at  $-70^\circ\text{C}$  until glutamate determination. For measurement of extracellular glutamate in anterior hypothalamus of rabbit brain, a CMA/12 microdialysis probe will be inserted into the guiding cannula. According to the methods described previously by Huang et al. (Huang et al., 2004) with slight modification, an equilibrium period of 120 min without sampling will be allowed after probe insertion. The microdialysis will be perfused at  $1.2 \mu\text{l}/\text{min}$ , and the dialysates will be sampled in microvials. The dialysates will be collected every 20 min in a CMA/140 fraction collector. Aliquots of dialysates ( $2 \mu\text{l}$ ) will be injected onto a CMA 600 Microdialysis analyzer for measurement of glutamate. The thermal experiments will be started after showing stabilization in four consecutive samples. In the present results, an equilibrium period of 4 h assures a stable level of the extracellular substance tested. Glutamate is enzymatically oxidized by glutamate oxidase. The hydrogen peroxide formed reacts with N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidine and 4-amino-antipyrine. This reaction is catalyzed by peroxidase and yields the red-violet colored quinonediimine. The rate of formation is measured photometrically at 546 nm and is proportional to the glutamate.

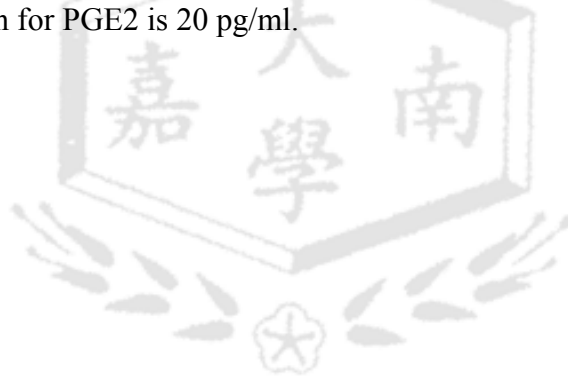


#### **(4) Determination of extracellular hydroxyl radical in the hypothalamus**

For measurement of extracellular hydroxyl radical in the POAH, the implanted probe will be perfused with artificial cerebrospinal fluid (aCSF) containing 10 mM salicylic acid by a high pressure pump (CMA/Microdialysis; RosLagsvägen, Stockholm, Sweden) at a flow rate of 1.2  $\mu\text{l}/\text{min}$  (Huang et al., 2006). It is known that salicylate can react with hydroxyl radical to generate stable dihydroxybenzoic acid (DHBA) derivatives, particular 2,3-DHBA, which can be used as in vivo indices of hydroxyl radical levels (Halliwell et al., 1991). The 2,3-DHBA in dialysates will be measured by high-performance liquid chromatography with a two-channel electrochemical detector (LC-4C, BAS, Bioanalytical System, West Lafayette, USA).

#### **(5) Measurement of prostaglandin E<sub>2</sub> in the hypothalamus**

For measurement of hypothalamic PGE<sub>2</sub>, the dialysis system will be connected to microdialysis pump and perfused with artificial cerebrospinal fluid at a flow rate of 1.2  $\mu\text{l}/\text{min}$ . The unanesthetized animals will be restrained in rabbit stocks for at least 120 min to achieve a stable dialysis level of PGE<sub>2</sub>. Dialysis samples from the POAH will be collected into microdialysis vial at 60 min intervals for 8 h and they will be stored at -80°C until analysed within 7 days. Immunoreactive PGE<sub>2</sub> concentrations in dialysates will be determined using commercially available enzyme immunoassay kits (Cayman Chemicals Co, Ann Arbor, MI). Triplicate aliquots of 50  $\mu\text{l}$  sample will be added to each well of plate and each sample will be assayed at a minimum of two dilutions. The limits of quantification for PGE<sub>2</sub> is 20 pg/ml.



### (三) 結果：

#### 一、腦室注射 IL-10 可抑制 LPS 發燒反應

如圖(一)及表(一)所示，靜脈注射 LPS(2 $\mu$ g/kg)可引起雙峯之發燒反應，分別在 90 分鐘及 180 分鐘，而且這些發燒反應都可被腦室注射 IL-10(10、50、100ng)所抑制，而且呈現劑量相關的抑制。

#### 二、IL-10 可抑制 LPS 在下視丘誘導麩胺酸及氫氧自由基之產生

圖(二)及圖(三)顯示，靜脈注射 LPS(2 $\mu$ g/kg)，仍可誘導腦部麩胺酸及氫氧自由基之產生，而且呈現雙峰之現象，分別在 90 分鐘及在 180 分鐘，其上升與肛溫是同步，在 LPS 注射前 1 小時，在腦室注射 IL-10(10、50、100ng)仍可有效抑制 LPS 在下視丘所產生之麩胺酸及氫氧自由基，並且呈現劑量相關的現象，如表(二)。

#### 三、IL-10 可抑制 LPS 在下視丘產生之 PGE<sub>2</sub>

圖(四)顯示先用 IL-10(10-100ng) 腦室注射，1 小時後再靜脈注射 LPS(2 $\mu$ g/kg) 可有效減少 LPS 刺激腦部產生之 PGE<sub>2</sub>。

### (四) 討論：

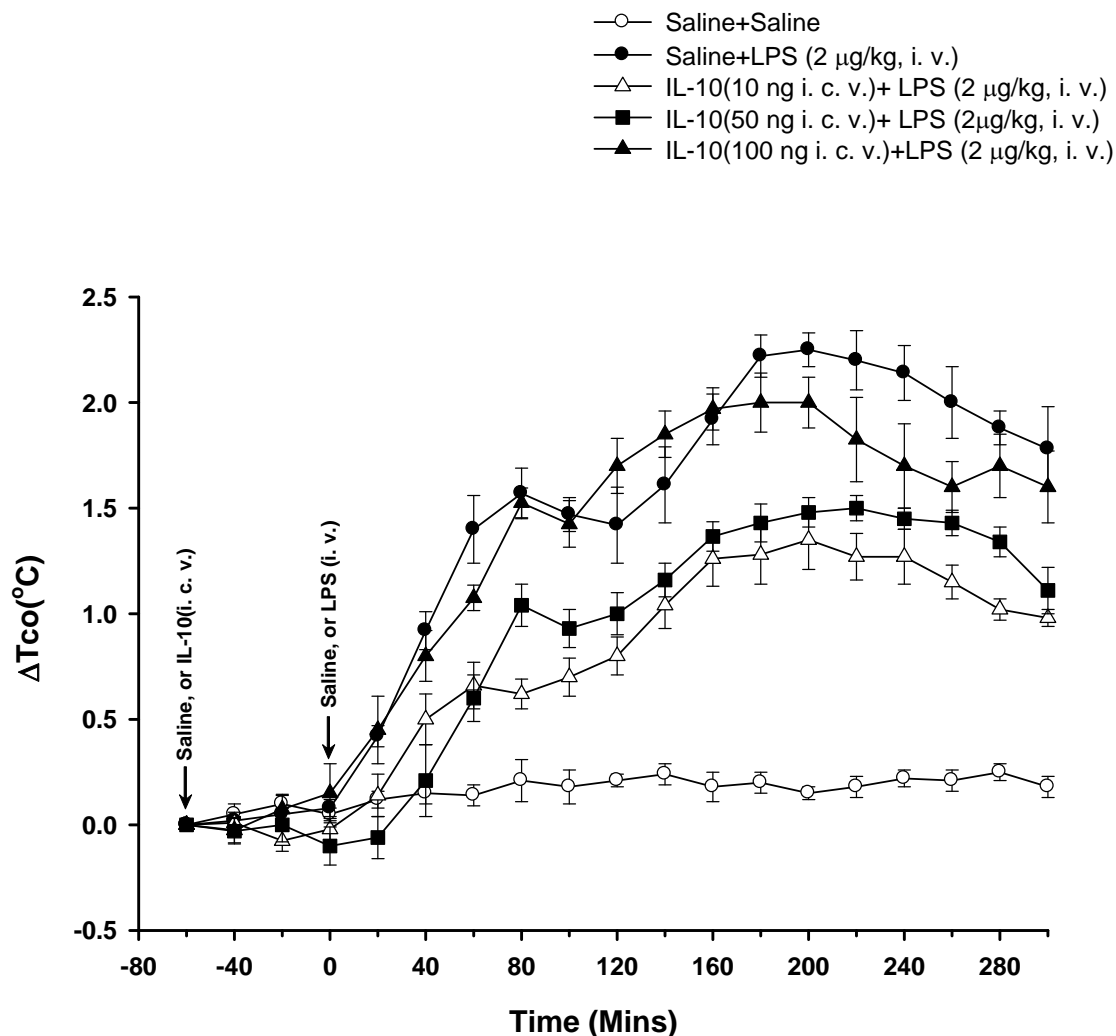
利用低劑量的致熱原如 LPS 可產生單峯之發燒反應，而如果使用高劑量之致熱原，則可產生雙峯之發燒 (Romanovsky, et al, 1998)，而且在很多動物都已經證實，包括兔子(Dinarelo, 2004) 及大白鼠 (Romanovsky, et al, 1998)。本實驗利用低劑量 (0.5 $\mu$ g/kg) 可產生單峯發燒，而高劑量(2-10  $\mu$ g/kg) 可產生雙峯之發燒，其結果是和上述作者之實驗相符，其高峯是出現在 LPS 打後 90 分鐘及 180 分鐘。

IL-10 是一種由 Th<sub>2</sub> 淋巴細胞所產生之抗發炎激素(Fiorentin, et al, 1989)，因其可抑制 THF-2、IL-1 及 IL-6 之產生，而具有抗發燒之作用。腦室注射 IL-10 可抑制腦室注射 LPS 所引起之發燒反應即在下視丘所產生之 IL-1 $\beta$  (Ledeboer, et al, 2002)。

本實驗最重要的發現主要是 IL-10 可透過抑制 LPS 在腦部誘導所產生之麩胺酸、氫氧自由基及 PGE<sub>2</sub>，而達到解熱的作用，而這在本人之其他實驗亦證實 baicalin (Huang et al, 2006)及 curcumin (Huang et al, 2008) 都可透過相同途徑來達到解熱的作用。總之，以 IL-10 1 小時前注射，可抑制 LPS 之發燒反應，而其機轉主要是透過抑制 LPS 所誘導下視丘之麩胺酸-氫氧自由基途徑及 PGE<sub>2</sub> 所產生，而達到解熱之作用。

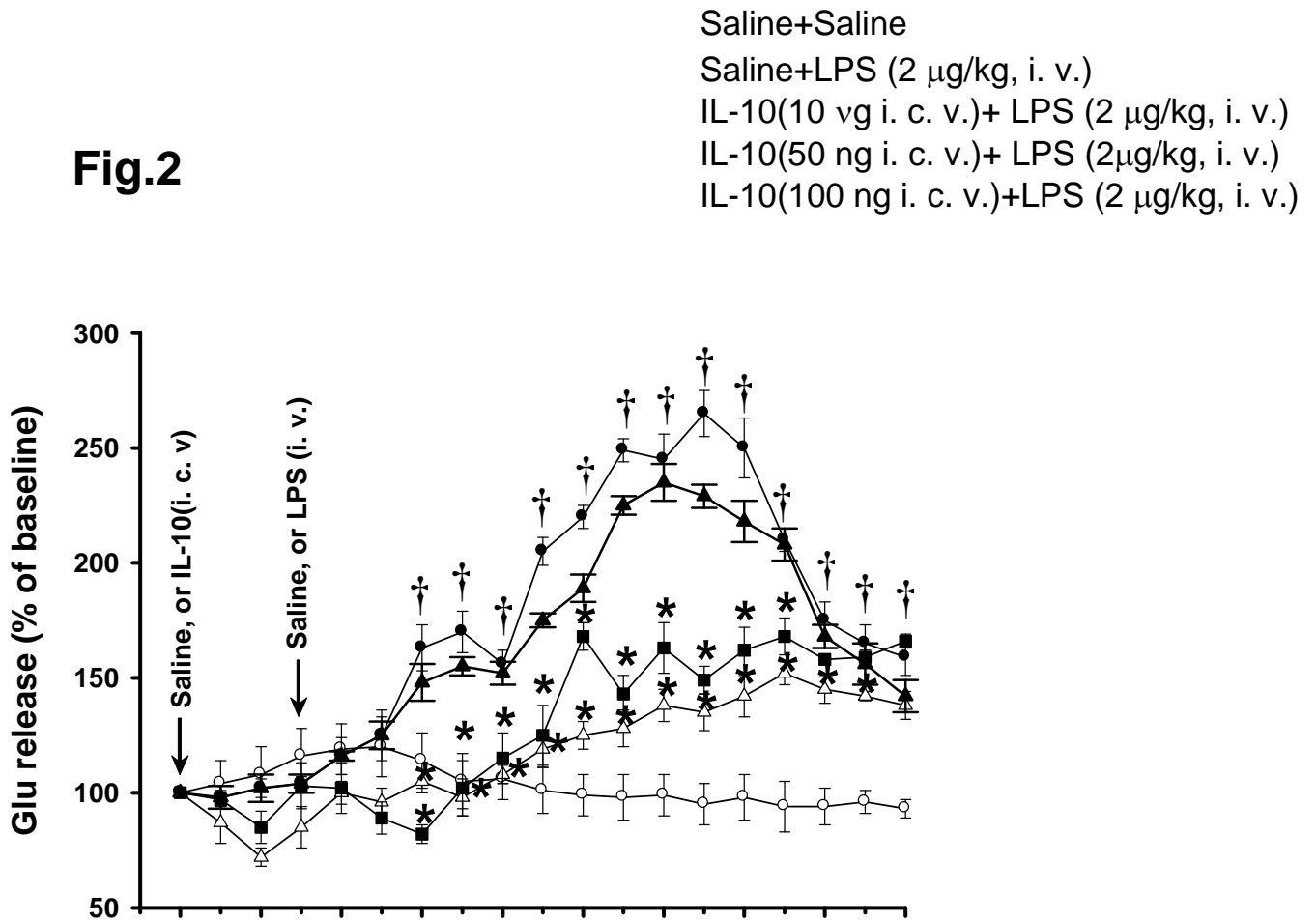
(五) 實驗部份：

Fig.1



**Fig. 1.** Mean  $\pm$  SEM changes in core temperature (Tco) in rabbits injected intracerebroventricular (i.c.v.) with either saline plus saline (i. v.)( $\circ$ ) (n=6), saline(i.c.v.) plus LPS (i. v.) (2  $\mu$ g/ kg) ( $\bullet$ ) (n=6), IL-10 at 100 ng(i.c.v.) plus LPS (2  $\mu$ g/kg) (i. v.) ( $\Delta$ ) (n=6), IL-10 at 50 ng(i.c.v.) plus LPS (2  $\mu$ g/kg) (i. v.) ( $\blacksquare$ ) (n=6), IL-10 at 10 ng(i.c.v.) plus LPS (2  $\mu$ g/kg) (i. v.) ( $\blacktriangle$ ) (n=8).  $\dagger P < 0.05$ , significantly different from corresponding control values (olive oil plus saline group) (ANOVA followed by Dunnett's test); \*  $P < 0.05$ , significantly different from corresponding control values (olive oil plus LPS group) (ANOVA followed by Dunnett's test).

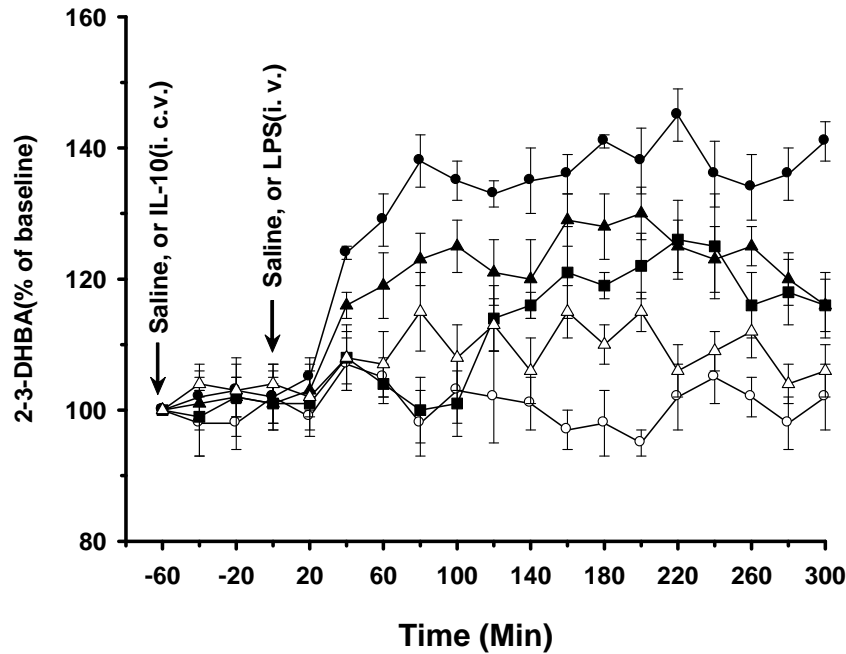
**Fig.2**



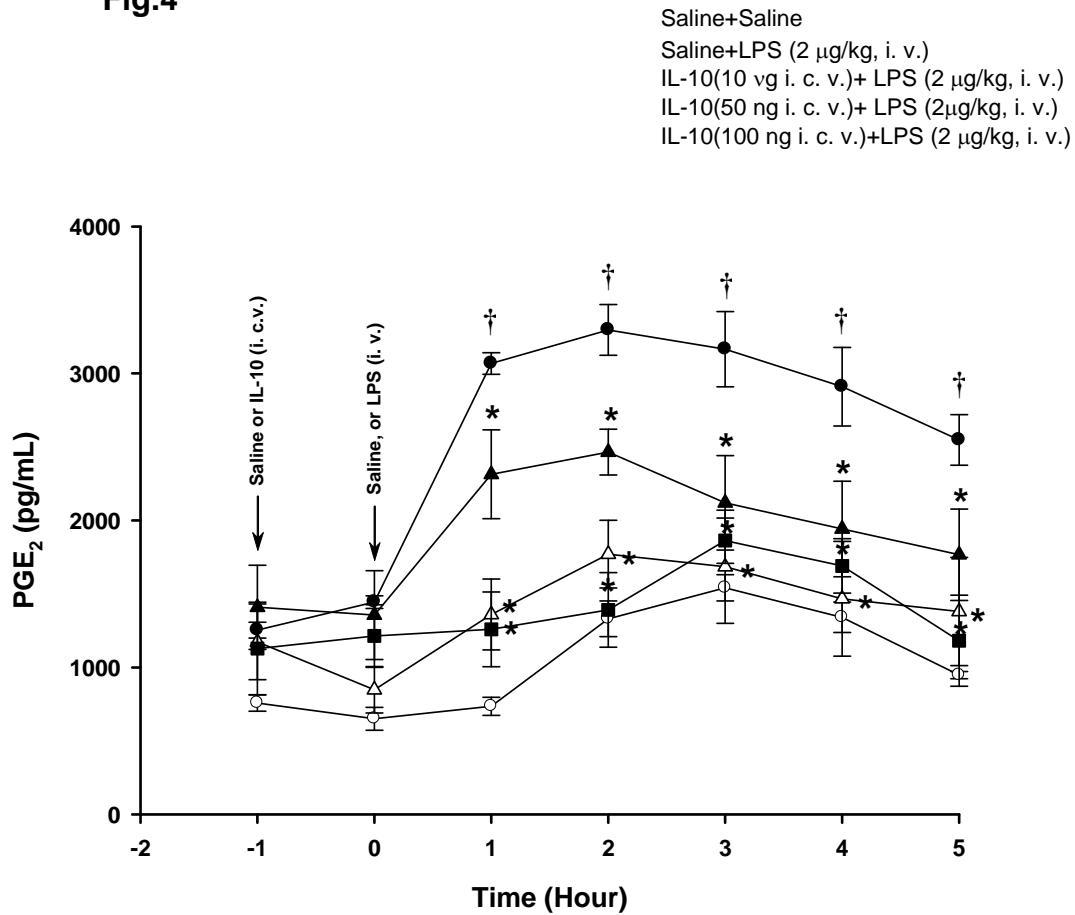
**Fig. 2.** Mean  $\pm$  SEM changes in glutamate (Glu) release in POAH with in rabbits injected intracerebroventricular (i.c.v.) with either saline plus saline (i. v.)( $\circ$ ) (n=6), saline(i.c.v.) plus LPS (i. v.) (2  $\mu$ g/ kg) ( $\bullet$ ) (n=6), IL-10 at 100 ng(i.c.v.) plus LPS (2  $\mu$ g/kg) (i. v.) ( $\Delta$ ) (n=6), IL-10 at 50 ng(i.c.v.) plus LPS (2  $\mu$ g/kg) (i. v.) ( $\blacksquare$ ) (n=6), IL-10 at 10 ng(i.c.v.) plus LPS (2  $\mu$ g/kg) (i. v.) ( $\blacktriangle$ ) (n=8)  $\dagger P < 0.05$ , significantly different from corresponding control values (olive oil plus saline group) (ANOVA followed by Dunnett's test); \*  $P < 0.05$ , significantly different from corresponding control values (olive oil plus LPS group) (ANOVA followed by Dunnett's test).

**Fig.3**

Saline+Saline  
Saline+LPS (2 µg/kg, i. v.)  
IL-10(10 ngi. c. v.)+ LPS (2 µg/kg, i. v.)  
IL-10(50 ng i. c. v.)+ LPS (2µg/kg, i. v.)  
IL-10(100 ng i. c. v.)+LPS (2 µg/kg, i. v.)



**Fig. 3.** Mean  $\pm$  SEM changes in hydroxyl radical generation in POAH with in rabbits injected intracerebroventricular (i.c.v.) with either saline plus saline (i. v.)(○) (n=6), saline(i.c.v.) plus LPS (i. v.) (2 µg/ kg) (●) (n=6), IL-10 at 100 ng(i.c.v.) plus LPS (2 µg/kg) (i. v.) (△) (n=6), IL-10 at 50 ng(i.c.v.) plus LPS (2 µg/kg) (i. v.) (■) (n=6), IL-10 at 10 ng(i.c.v.) plus LPS (2 µg/kg) (i. v.) (▲) (n=8) †P < 0.05, significantly different from corresponding control values (olive oil plus saline group) (ANOVA followed by Dunnett's test); \* P < 0.05, significantly different from corresponding control values (olive oil plus LPS group) (ANOVA followed by Dunnett's test).

**Fig.4**

**Fig. 4.** Mean  $\pm$  SEM changes in prostaglandin E<sub>2</sub> generation in POAH with in rabbits injected intracerebroventricular (i.c.v.) with either saline plus saline (i. v.)(○) (n=6), saline(i.c.v.) plus LPS (i. v.) (2 µg/ kg) (●) (n=6), IL-10 at 100 ng(i.c.v.) plus LPS (2 µg/kg) (i. v.) (△) (n=6), IL-10 at 50 ng(i.c.v.) plus LPS (2 µg/kg) (i. v.) (■) (n=6), IL-10 at 10 ng(i.c.v.) plus LPS (2 µg/kg) (i. v.) (▲) (n=8) †P < 0.05, significantly different from corresponding control values (olive oil plus saline group) (ANOVA followed by Dunnett's test); \* P < 0.05, significantly different from corresponding control values (olive oil plus LPS group) (ANOVA followed by Dunnett's test).

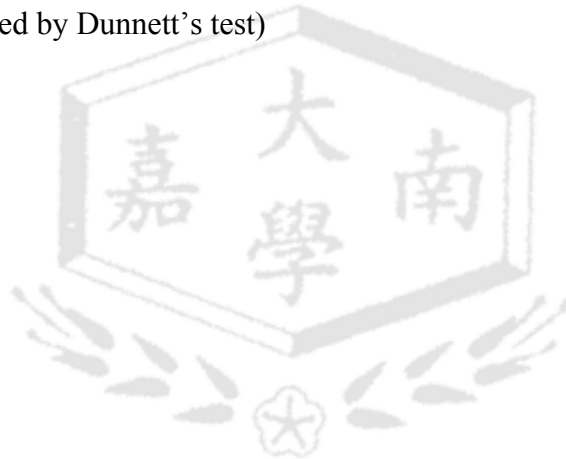
**Table 1.** Effects of central administration of IL-10 one hour before the LPS injection on the febrile response to intravenous injection of LPS in rabbits.

Treatments	Fever index (FI, °C · h)
Saline + Saline	FI = 0.98 ± 0.07
Saline + LPS (2 µg/kg)	FI = 8.15 ± 0.11 <sup>†</sup>
IL-10(10 ng, i.c.v.) + LPS (2 µg/kg)	FI = 7.38 ± 0.15 <sup>*</sup>
IL-10(50 ng, i.c.v.) + LPS (2 µg/kg)	FI = 4.49 ± 0.13 <sup>*</sup>
IL-10(100 ng, i.c.v.)+ LPS (2 µg/kg)	FI = 3.87 ± 0.11 <sup>*</sup>

The values are means ± SEM of 8 rabbits per group. FI represent fever index for 6 h experimental observation.

<sup>†</sup>Significantly different from corresponding control value (vehicle plus vehicle group) (P < 0.05; two way analysis of variance followed by Dunnett's test);

<sup>\*</sup>Significantly different from corresponding control value (vehicle plus LPS) (P < 0.05; two way analysis of variance followed by Dunnett's test)



**Table 2.** Effects of central administration of IL-10 one hour before the LPS injection on peak Tco, glutamate, and hydroxyl radicals elevation in POAH in response to LPS in rabbits.

Treatments	Response	
	early phase	late phase
	Peak Tco	(°C)
Saline + Saline	0.18± 0.005	0.23 ± 0.08
Saline + LPS(2 µg/kg)	1.57 ± 0.12 <sup>†</sup>	2.25± 0.08 <sup>†</sup>
IL-10 (10 ng, i.c.v.) + LPS (2 µg/kg)	1.52 ± 0.17	1.85 ± 0.12 <sup>*</sup>
IL-10 (50 ng, i.c.v.) + LPS (2 µg/kg)	0.67± 0.07 <sup>*</sup>	1.35 ± 0.11 <sup>*</sup>
IL-10 (100 ng, i.c.v.) + LPS (2 µg/kg)	0.62 ± 0.15 <sup>*</sup>	1.15 ± 0.13 <sup>*</sup>
	% of mean basal levels of glutamate	
Saline + Saline	104 ± 5	95 ± 9
Saline + LPS(2 µg/kg)	168± 9 <sup>†</sup>	263± 7 <sup>†</sup>
IL-10 (10 ng, i.c.v.) + LPS (2 µg/kg)	98 ± 8 <sup>*</sup>	226± 7 <sup>*</sup>
IL-10 (50 ng, i.c.v.) + LPS (2 µg/kg)	102± 12 <sup>*</sup>	149± 6 <sup>*</sup>
IL-10 (100 ng, i.c.v.) + LPS (2 µg/kg)	155± 4 <sup>*</sup>	135± 8 <sup>*</sup>
	% of mean basal levels of hydroxyl radical	
Saline + Saline	102 ± 3	99 ± 3
Saline + LPS(2 µg/kg)	138± 6 <sup>†</sup>	151 ± 2 <sup>†</sup>
IL-10 (10 ng, i.c.v.) + LPS (2 µg/kg)	123 ± 4	136 ± 5 <sup>*</sup>
IL-10 (50 ng, i.c.v.) + LPS (2 µg/kg)	115 ± 9 <sup>*</sup>	115 ± 4 <sup>*</sup>
IL-10 (100 ng, i.c.v.) + LPS (2 µg/kg)	105 ± 4 <sup>*</sup>	108 ± 3 <sup>*</sup>

The values are means ± SEM of 8 rabbits per group. Rabbits injected LPS (2µg/kg) produced a biphasic fever which peaked at 80 mins (early phase) and 200 mins (late phase) after LPS injection.

<sup>†</sup>Significantly different from corresponding control value (vehicle plus vehicle group) (P < 0.05; two way analysis of variance followed by Dunnett's test);

<sup>\*</sup> Significantly different from corresponding control value (vehicle plus LPS group) (P < 0.05; two way analysis of variance followed by Dunnett's test).



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