行政院國家科學委員會專題研究計畫成果報告

葛根素對大鼠體溫調節作用及下視丘五羥色胺酸濃度變化之影響

Effects of Puerarin on Thermoregulatory Response and Hypothalamic Serotonin Release in Rats

† 八十六年度及以前的一般國科會專題計畫(不含產學合作研究計畫)亦可選擇適用，惟較特殊的計畫如國科會規劃案等，請先洽得國科會各學術處同意。

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一、中文摘要

葛根素 (puerarin) 為野葛 (*Pueraria lobata*) 主要活性成分之一，已知為 β-adren-ergic receptor 阻斷劑，具有解痙、抗心率不整、降血壓等作用，此外還具有解熱等作用，但有關 puerarin 降溫作用之機轉至今仍並未見有研究報告提出；因此本研究擬就 puerarin 對清醒大鼠之降溫作用機轉進行探討。研究結果顯示，由側腦室給予 puerarin (100μg/kg, intracerebro-ventricular injection; i.c.v.) 可引起室溫下 (24±1℃) 正常清醒大鼠體溫降低作用，且腹腔給予 puerarin (5-30mg/kg, intraperi-toneal admi-nistration; i.p.) 可引起一具劑量依存性之體溫降低作用，並同步降低大鼠下視丘 serotonin (5-HT) 之濃度；此降溫作用會因側腦室給予 serotonin neuro-toxin 5,7-dihydroxytryptamine (5,7-DHT, 200μg/10μl, i.c.v.) 或皮下給予5-HT1A 受體之拮抗劑 (-)-pindolol (0.05, 0.5mg/kg, subcutaneously injection; s.c.)所減弱，但可被皮下給予 5-HT1A 受體之致效劑 8-hydroxy-dipropylaminotetralin (8-OH-DPAT; 0.05 mg/kg; s.c.) 所加強。此外，puerarin 誘發之降溫作用亦會被5-HT2 受體之致效劑 (±)-2,5-dimethoxy-4-iodoamphetamine (DOI; 5, 10μg/10μl; i.c.v.; 0.5, 1mg/kg; i.p.)、quipazine (0.1, 1mg/kg; i.p.) 所拮抗，或為 5-HT2 受體之拮抗劑 pirenperone (0.2mg/kg; s.c.)、ketanserin (1mg/kg; i.p.) 所加強。由此可推知 puerarin 可能藉由降低下視丘 serotonin 之濃度，並作用於 postsynaptic serotonin 受體，致活 5-HT1A 受體及阻斷 5-HT2 受體，而達到降低體溫之作用。

關鍵詞：葛根素、降溫作用、五羥色胺酸

Abstract

Puerarin is an isoflavone compound isolated from *Pueraria lobata.* Puerarin, a β-adrenergic receptor blocker, possesses anti-convulsive, antiarrhythmic and antihyper-tension effects. It also reduces 2,4-dinitro-phenol-induced hyperthermia. However, the effects of puerarin on normal body tempe-rature are unknown. On this account, in the present study, experiments were carried out to assess the mechanism of puerarin induced-hypothermia in unanesthetized rats.

Puerarin (100μg/10μl, i.c.v.; 5-30mg/kg, i.p.) caused a dose-related fall in both colonic temperature and the 5-HT release in the hypothalamus at room temperature. Puerarin induced hypothermia was attenuated by pretreatment with 5,7-dihydroxytryptamine (5,7-DHT; a serotonin neurotoxin, 200 μg/10μl; i.c.v., one week ago), or (-)-pindolol (a $5\text{-HT}_{1A}/\beta$ adrenoceptor antagonist; 0.05, 0.5mg/kg; s.c.) but potentiated by (±)-8–hydroxydiopropylaminoteralin (8-OH-DPAT; a 5-HT_{1A} receptor agonist; 0.05mg/kg; s.c.). In addition, the puerarin induced hypothermia was attenuated by (±)-2,5-dimethoxy-4-iodoamphetamine (DOI; 5-HT_2 receptor agonist; 5, 10μg/10μl; i.c.v.; 0.5, 1mg/kg; i.p.) or quipazine (a 5-HT_2 receptor agonist; 0.5, 1mg/kg; i.p.), but potentiated by ketanserin (5-HT_2 receptor antagonist; 1mg/kg; i.p.) or pirenperone (5-HT_2 receptor antagonist; 0.2mg/kg; s.c.). These results

indicate that puerarin may act through 5-HT_{1A} receptor activation or 5-HT_2 receptor antagonism within the brain to induce its hypothermia.

Keywords: Puerarin, Hypothermia, Hypothalamus, Serotonin

二、緣由與目的

葛根素 (puerarin) 為葛根之主要成分之一，屬 isoflavonoids；近年來藥理研究發現其為一β-adrenoreceptor 之阻斷劑[1]，對腦內之 benzodiazepine receptor 亦具致效作用[2]；此外 puerarin 還具有解酒[3]、解痙[4]、促進腦及心臟血流[5-8]，降血壓[9,10]、抗心率不整[11]、降低 2,4-dinitrophenol 誘發之高溫[12]等作用。然有關 puerarin 解熱降溫作用之機轉至今仍並未見有研究報告提出，本實驗室於八十八年度年度國科會專題研究計畫中，首先就 puerarin 對熱原性發燒大鼠之解熱作用進行探討，初步研究結果顯示室溫下 puerarin (20mg/kg, i.p.) 除可降低細菌內毒素 lipoplysaccharide (LPS) 誘發高溫大鼠之直腸溫度（肛溫）外，亦可明顯降低正常大鼠之直腸溫度。

1957 年 Brodie & Shore 與 1961 年 Von Euler 先後提出下視丘 可能在體溫調節作用上扮演一控制的角色[13]，及腦內單胺神經系統可能參與下視丘體溫恆定 (thermostat) 設定的機制後[14]，於 1980 年 Myers 等學者發現下視丘 serotonin (5-HT) 之含量與熱的產生有關[15]，1991 年 Gorden 進一步報告指出當動物下視丘之 5-HT 活性增加時可使代謝產熱增加散熱減少 (表皮血管收縮)，反之，當動物下視丘 5-HT 活性降低會使代謝產熱減少散熱增加 (表皮血管擴張，呼吸散熱率增加)[16]，可知下視丘之 serotoninergic system 在體溫調節系統中扮演一重要之角色。因此本研究擬繼續就 puerarin 對室溫下正常大鼠直腸溫度變化及其與下視丘 serotoninergic system 之關係進行探討。

三、結果與討論

實驗結果發現腹腔給予 puerarin (5-30mg/kg) 對室溫下正常大鼠之體溫具明顯之降溫作用，給藥後約 120 分鐘體溫降至最低，其後則慢慢回復，持續時間則會隨劑量增加而延長 (Fig.1)，而側腦室給予 puerarin (100μg/rat) 對室溫下正常大鼠之體溫也具明顯之降溫作用(Fig.2)。

為探討 puerarin 之降溫作用與體溫調節中樞 (下視丘) serotoninergic system 間之關係，本研究利用微透析法配合高壓液相層析儀，對 puerarin 對於室溫下正常清醒大鼠肛溫與下視丘 5-HT 濃度變化進行同步偵測，實驗結果發現當 puerarin 降低大鼠肛溫時，其下視丘 5-HT 之濃度亦有降低之現象 (Table 1)；故 puerarin 之降溫作用可能與降低下視丘 5-HT 之濃度有關。

5,7-dihydroxytryptamine (5,7-DHT) 為 5-HT neurons之 neurotoxin[17-19]，是神經科學研究上常用之工具藥，由大鼠側腦室一次給予 5,7-DHT，七天後可明顯降低下視丘 5-HT 濃度，但不會影響下視丘 dopamine 之濃度，且對大鼠之體溫並無明顯之影響[20]。由於 puerarin 之降溫作用，會因 5,7-DHT 破壞腦中 5-HT neurons 而減弱 (Table 2)；可推知突觸後 serotoninergic receptors 亦可能參與 puerarin 降溫作用之產生。

目前已知腦中至少存在有七種不同之 serotoninergic receptor (5-$HT_{1A/B/D/E/F}$, 5-$HT_{2A/B/C}$, 5-HT_3, 5-HT_4, 5-HT_5, 5-HT_6 及 5-HT_7)[21]。相關研究發現 serotoninergic receptors 與體溫調節作用之關係會因使用動物品種、藥物劑量大小、給藥途徑及部位之不同等而有所差異。(-)-pindolol 為 5-HT_{1A} 受體及 β-adrenoceptor 之拮抗劑，可拮抗 5-HT_{1A} 受體之致效劑 (8-OH-DPAT, gepirone, (+)S-20499) 所誘發之降溫作用[22]；8-OH-DPAT 為突觸前及突觸後 5-HT_{1A} 受體之致效劑[23,24]，腹腔或皮下給予可引起明顯之降溫作用[25-28]，並降低腦中 5-HT 之釋放[29]；近來研究指出 8-OH-DPAT 降溫作用之機轉大鼠與鼷鼠並不相同，在大鼠

8-OH-DPAT 之降溫作用不會因 5,7-DHT 前處理破壞 5-HT neurons 或利用 *p*-chlorophenylalanine (*p*-CPA; 5-HT synthesis inhibitor) 排空腦內 5-HT 含量而消失, 亦不因給予選擇性之 5-HT uptake 抑制劑、5-HT precursor 或 5-HT releasing agent 提高腦內 serotonin 之釋放所影響, 故其對大鼠降溫作用是藉由興奮突觸後 5-HT_{1A} 受體所達成[30,31]；而鼷鼠則剛好相反是藉由興奮突觸前 5-HT_{1A} 受體所致[31,32]。實驗結果發現 puerarin 誘發之降溫作用可被 5-HT_{1A} 受體之拮抗劑 (-)-pindolol 所拮抗 (Fig.3)，或為 5-HT_{1A} 受體之致效劑 8-OH-DPAT 所加強 (Fig.4)；故可推知 puerarin 對室溫下正常大鼠之降溫作用可能是藉由致效突觸後 5-HT_{1A} 受體所致。

其次, 全身性給予 $5\text{-HT}_{2A/C}$ 受體之致效劑 DOI、MK-212 或 quipazine 等均會引起體溫上升之作用[27,33-35], 且此升溫作用可為事先給予 $5\text{-HT}_{2A/C}$ 受體之拮抗劑 ketanserin、LY53857、mianserin、ritanserin 或 $5\text{-HT}_{1A/2}$ 及 D_2 受體之拮抗劑 spiperone 所阻斷[36,37]，而 5-HT_2 受體之拮抗劑 ketanserin 或 pirenperone 單獨給藥時則具降溫之作用[38]，顯示 5-HT_2 受體在體溫調節作用上扮演一與 5-HT_{1A} 受體相反之升溫的角色；進一步的研究發現 DOI 不論中樞或周邊給予均可誘發大鼠之升溫作用[20]，且其升溫作用不受 $5\text{-HT}_{1A/1B/2C}$ 受體及 β-adrenoceptor 之拮抗劑 propranolol 與 5-HT_3 受體之拮抗劑 MDL-72222 或 ondansetron 所影響, 可知 DOI 是藉由興奮突觸後 5-HT_{2A} receptor 誘發大鼠之升溫作用[37]；而 quipazine 不僅為 $5\text{-HT}_{1B/1C/2}$ 受體之致效劑亦為 5-HT_3 受體之拮抗劑[39] 與 5-HT-uptake 之抑制劑[40]，可增加突觸間 serotonin 之濃度[41-43]，其誘發之升溫作用亦是藉由興奮突觸後 5-HT_2 受體所致[44,45]。實驗結果發現 puerarin 誘發之降溫作用可被腹腔給予 pirenperone、ketanserin 之 5-HT_2 受體拮抗劑所加強 (Fig.5,6)，或為腹腔或側腦室給予 DOI 及腹腔給予 quipazine 之 5-HT_2 受體致效劑所拮抗 (Fig.7-9)；可知 puerarin 對室溫下正常大鼠之降溫作用可能與阻斷突觸後 5-HT_2 受體有關。

綜合上述實驗結果，可知 puerarin 可能藉由降低下視丘 5-HT 之濃度，並作用於 postsynaptic serotonin recepter，致活 5-HT_{1A} 受體及阻斷 5-HT_2 受體，而達到降低體溫之作用。

四、計畫成果自評

本研究計畫案計畫書中擬進行之各項實驗除部分藥物給藥方式與劑量，依實際實驗狀況略有修正更動外，均已完成。

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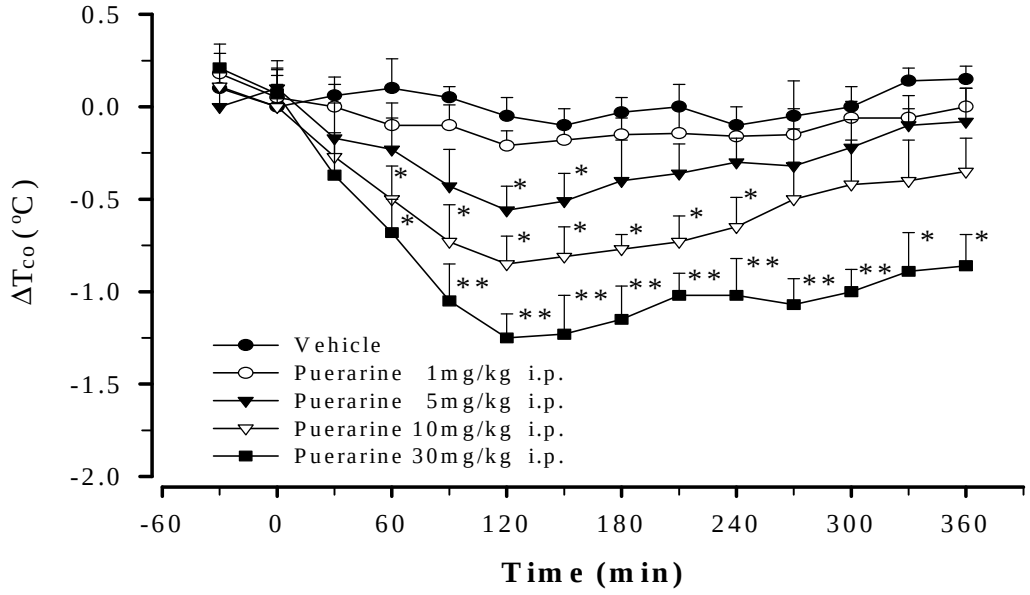

Fig. 1 Time course of the effects of intraperitoneal administration of puerarin on colonic temperature in rats. Puerarin (1, 5, 10, 30mg/kg, i.p.) was injected at 0 min. The colonic temperature of vehicle-injected rats was 37.82±0.20°C at time 0 min. Δ, denote the difference between the control value before injected and exchange after injected. The value are mean±SEM of 8-12 rats per group.*P<0.05, **P<0.01, significantly different from corresponding control value (vehicle group), ANOVA.

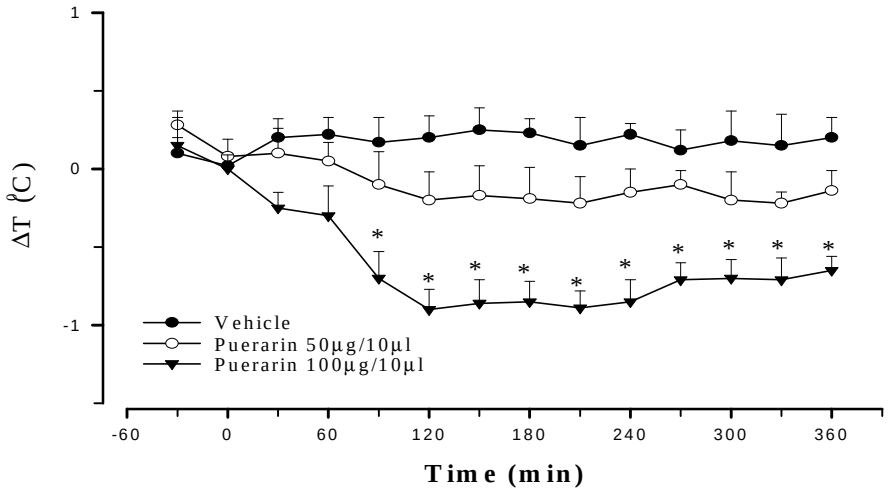

Fig. 2 Time course of the effects of intracerebroventricular administration of puerarin on colonic temperature in rats. Puerarin (50, 100μg/kg, i.c.v.) was injected at 0 min. The colonic temperature of vehicle-injected rats was 37.14±0.15°C at time 0 min. Δ, denote the difference between the control value before injected and exchange after injected. The value are mean±SEM of 8 rats per group.*P<0.05, significantly different from corresponding control value (vehicle group), ANOVA.

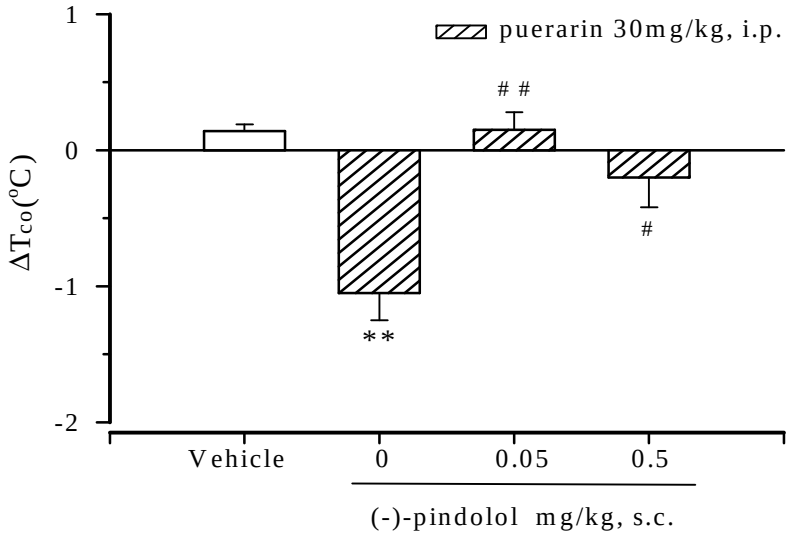

Fig. 3 Effects of (-)-pindolol (0.05, 0.5mg/kg; s.c.) on the hypothermic response of puerarin in rats. Puerarin was injected 30 mins before (-)-pindolol (0.05, 0.5mg/kg) subcutaneously injected (s.c.) The value are mean±SEM of 8 rats per group. Δ, denote the difference between the control value before 120 mins after the start of puerarin injected. **P<0.01, significantly different from the corresponding control value (vehicle group), ANOVA. $^{\#}$P<0.05, $^{\#\#}$P<0.01 significantly different from corresponding control value (puerarin group), ANOVA.

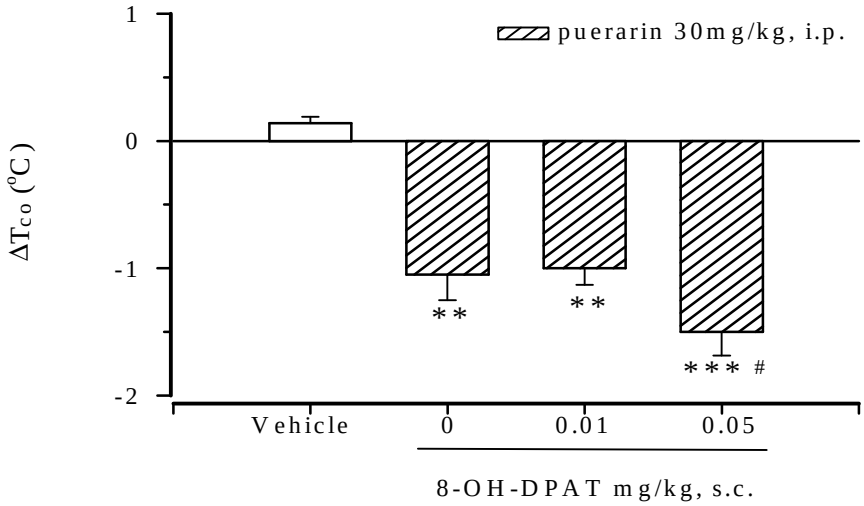

Fig. 4 Effects of 8-OH-DPAT (0.01, 0.05mg/kg, s.c.) on the hypothermic response of puerarin in rats. Puerarin was injected 100 mins before 8-OH-DPAT (0.01, 0.05mg/kg) subcutaneously injected (s.c.) The value are mean±SEM of 8 rats per group. Δ, denote the difference between the control value before 120 mins after the start of puerarin injected. **P<0.01, ***P<0.001 significantly different from the corresponding control value (vehicle group), ANOVA. #P<0.05 significantly different from corresponding control value (puerarin group), ANOVA.

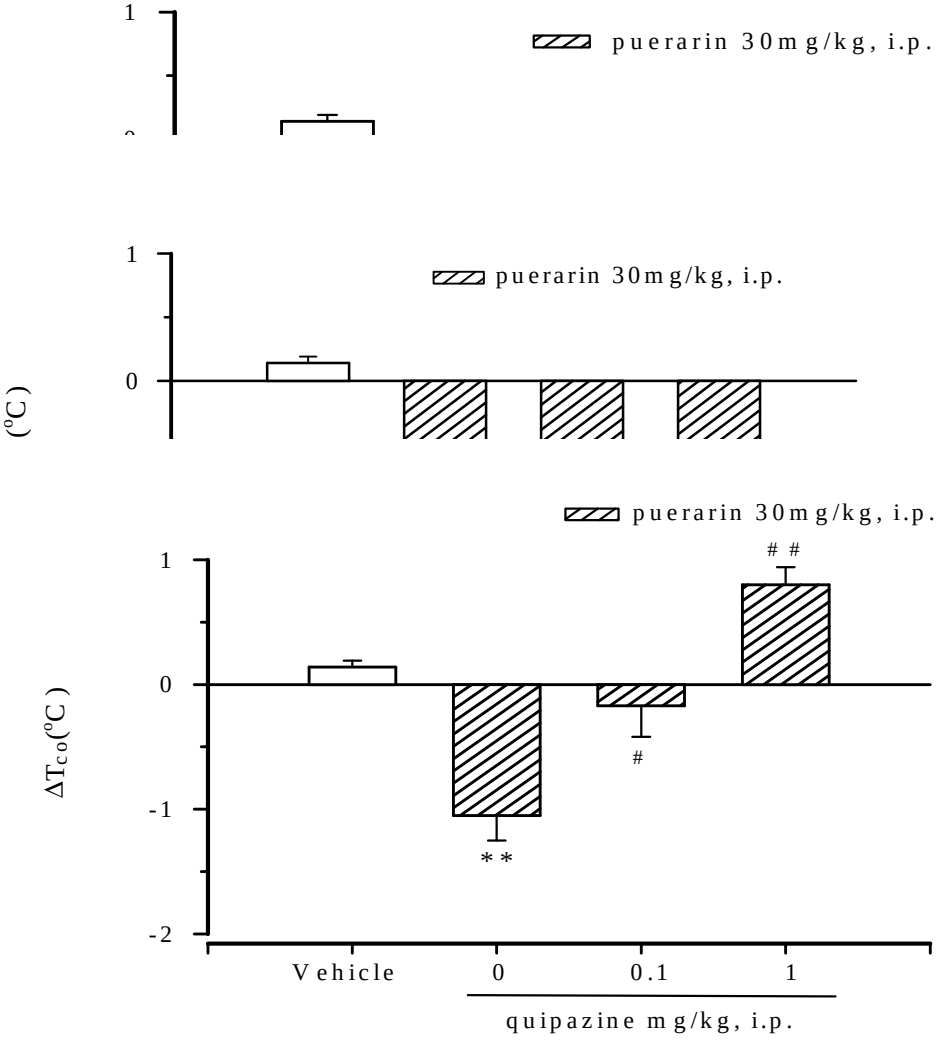

Fig. 7 Effects of quipazine (0.1, 1mg/kg, i.p.) on the hypothermic response of puerarin in rats. Puerarin was injected 60 mins before quipazine (0.1, 1mg/kg) intraperitoneal injected (i.p.) The value are mean±SEM of 8 rats per group. Δ, denote the difference between the control value before 120 mins after the start of puerarin injected. **P<0.01 significantly different from the corresponding control value (vehicle group), ANOVA. $^{\#}$P<0.05, $^{\#\#}$P<0.01 significantly different from corresponding control value (puerarin group), ANOVA.

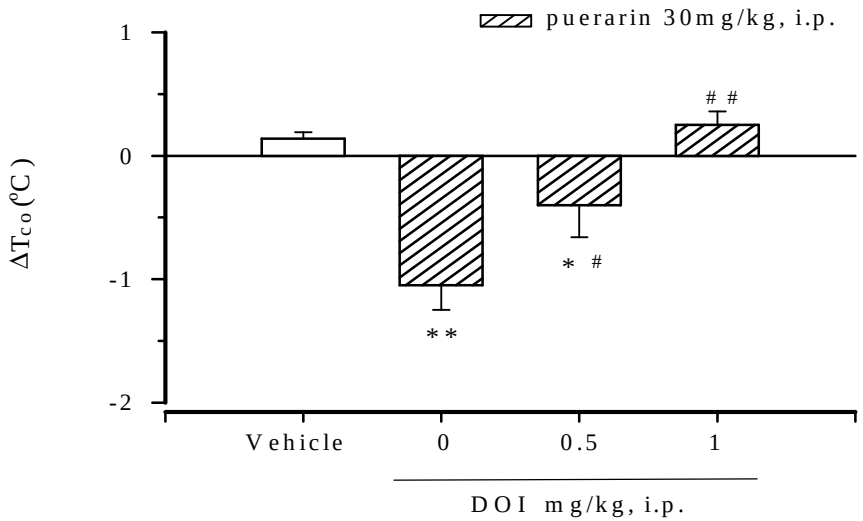

Fig. 8 Effects of DOI (0.5, 1mg/kg, i.p.) on the hypothermic response of puerarin in rats Puerarin was injected 90 mins before DOI (0.5, 1mg/kg) intraperitoneal injected (i.p.) The value are mean±SEM of 8 rats per group. Δ, denote the difference between the control value 120 mins after the start of puerarin injected. *P<0.05, **P<0.01, significantly different from the corresponding control value (vehicle group), ANOVA. $^{\#}$P<0.05, $^{\#\#}$P<0.01 significantly different from corresponding control value (puerarin group), ANOVA

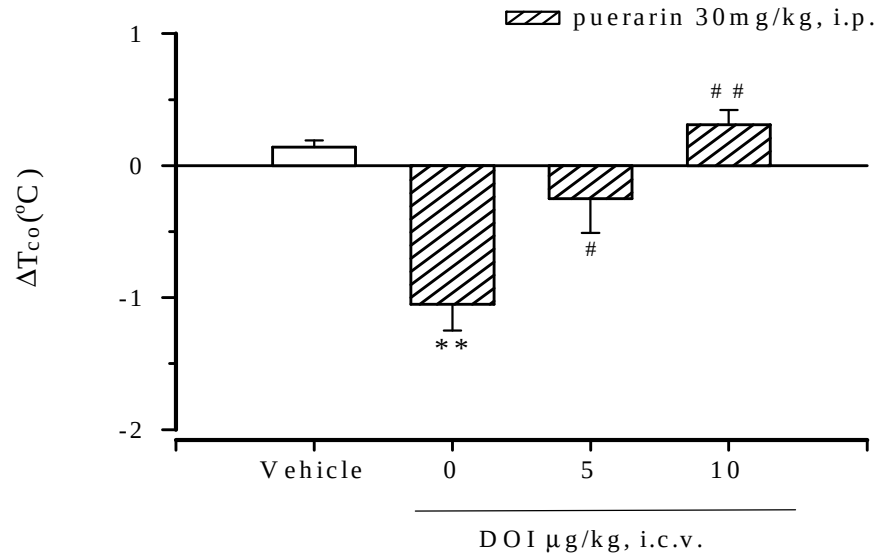

Fig. 9 Effects of DOI (5, 10μg/10μl, i.c.v.) on the hypothermic response of puerarin in rats. Puerarin was injected 90 mins before DOI (5,10μg/10μl) intracrerbroventricular injected (i.c.v.) The value are mean±SEM of 8 rats per group. Δ, denote the difference between the control value 120 mins after the start of puerarin injected. *P<0.05, **P<0.01, significantly different from the corresponding control value (vehicle group), ANOVA. $^{\#}$P<0.05, $^{\#\#}$P<0.01 significantly different from corresponding control value (puerarin group), ANOVA

Table 1. The effect of puerarin on the hypothalamic serotonin (5-HT) release in the normal rats.

Treatments (i.p.)	Δ Colonic temperature (°C)	Hypothalamic 5-HT release (% baseline)
Vehicle	0.14±0.05	99.12±34.25
Puerarin		
10mg/kg	-0.78±0.21*	52.14±35.23*
30mg/kg	-1.05±0.20**	25.12±31.22**

The value are mean±SEM of 5 rats per group. The vehicle-treated control value for extracellular 5-HT release in the hypothalamus are 1.58±0.65pg/18μl/30 mins. Δ, denote the difference between the control value before 120 mins after the start of puerarin injected. *P<0.05, **P<0.01 significantly different from the corresponding control value (vehicle group), ANOVA.

Table 2. The effects of puerarin on the colonic temperature in rats treated by the 5,7-DHT.

Treatment	Changes in colonic temperature (Δ℃)	
	Normal	5,7-DHT
Vehicle	0.14±0.05	0.20±0.14
puerarin		
10mg/kg (i.p.)	-0.78±0.21*	-0.21±0.11$^{\#}$
30mg/kg (i.p.)	-1.05±0.20**	-0.53±0.13*$^{\#}$

The value are mean±SEM from 8 rats per group. The control value for colonic temperature are 37.8±0.12℃ and 38.1±0.14℃ for normal and 5,7-DHT-treated rats, respectively. Δ, denote the difference between the control value before 120 mins after the start of puerarin injected. *P<0.05, **P<0.01 significantly different from corresponding control value (vehicle group), ANOVA. $^{\#}$P<0.05 significantly different from corresponding control value (normal group), ANOVA.