Full Paper

Puerarin Acts Through Brain Serotonergic Mechanisms to Induce Thermal Effects

Fu-Shin Chueh¹, Ching-Ping Chang², Chung-Ching Chio^{3,*}, and Mao-Tsun Lin²¹Department of Applied Life Science, Taichung Healthcare and Management University, Taichung, Taiwan 413²Department of Medical Research, Chi-Mei Medical Center, Tainan, Taiwan 710³Department of Neurosurgery, Chi-Mei Medical Center, Tainan, Taiwan 710

Received July 1, 2004; Accepted October 4, 2004

Abstract. The present study was attempted to investigate the effect of puerarin, an isoflavone compound isolated from *Pueraria lobata*, on both the basal body temperature and pyrogenic fever in unanesthetized, restrained rats. Intraperitoneal administration of puerarin or crude extracts of *Pueraria lobata* elicited hypothermia. Direct administration of a small amount of puerarin into the lateral cerebral ventricle produced the same extent of hypothermia. Systemic or central administration of puerarin causes a decrease in both colonic temperature and hypothalamic 5-HT efflux in rats. The puerarin-induced hypothermia and decreased 5-HT efflux in the hypothalamus were attenuated by selective depletion of hypothalamic 5-HT produced by intracerebroventricular injection of 5,7-dihydroxytryptamine. Furthermore, the puerarin-induced hypothermia was almost completely abolished by treatment with a 5-HT_{2A}-receptor agonist (DOI or quipazine) or a 5-HT_{1A}-receptor antagonist [(–)-pindolol]. A 5-HT_{2A}-receptor antagonist (ketanserin) or a 5-HT_{1A}-receptor agonist (8-OH-DPAT) had additive effects with puerarin. Intracerebroventricular administration of interleukin-1 caused an increase in both colonic temperature and hypothalamic 5-HT efflux. The interleukin-1-induced hyperthermia and increased 5-HT efflux in the hypothalamus were attenuated by treatment with systemic administration of puerarin. The data indicate that puerarin exerts its hypothermic and antipyretic effects by activating 5-HT_{1A} receptor and/or antagonizing 5-HT_{2A} receptors in the hypothalamus.

Keywords: fever, hypothermia, serotonin, puerarin, hypothalamus

Introduction

Puerarin [4*H*-1-benzopyran-4-one,8- β -D-glucopyranosyl-7-hydroxy-3-(4-hydroxy-phenyl), C₂₁H₂₀O₉] is one of 3 major isoflavonoid compounds isolated from *Pueraria lobata*, an edible vine used widely in China for various purposes. It has been shown to be able to block the beta adrenoceptors of isolated organs and the whole animal (1). In addition, it possesses anti-convulsive actions (2); suppresses alcohol intake (3), myocardial reperfusion injury (4), and arterial hypertension (5); and has anti-arrhythmic actions (6). In particular, the hyperthermia produced by 2,4-dinitrophenol can be attenuated by puerarin (7). However, the mechanisms underlying the thermal effects exerted by

puerarin remain unclear.

Evidence has accumulated to indicate that the 5-HT_{2A}- and 5-HT_{1A}-receptor activation in the hypothalamus mediate the hyperthermic and hypothermic action, respectively, in rats (8, 9). This raises the hypothesis that puerarin acts through a 5-HT_{2A}- and/or 5-HT_{1A}-receptor mechanism to exert its thermal effect.

To deal with the hypothesis, in the present study, we firstly attempted to assess whether puerarin is able to affect basal body temperature and/or pyrogenic fever in unanesthetized rats. Then, we assessed whether the thermal effects exerted by puerarin can be altered by pretreatment with 5,7-dihydroxytryptamine (5,7-DHT), a 5HT-depleting agent (10, 11); (–)-pindolol, a 5-HT_{1A}-receptor antagonist; (±)-8-hydroxydiopropylaminotetralin (8-OH-DPAT), a 5-HT_{1A}-receptor agonist (12, 13); (±)-2,5-dimethoxy-4-iodoamphetamine (DOI), a 5-HT_{2A}-

*Corresponding author. FAX: +886-6-2517850
E-mail: 891201@mail.chimei.org.tw

receptor agonist; or ketanserin, a 5-HT_{2A}-receptor antagonist (14–16).

Materials and Methods

Animals

Male Sprague-Dawley rats, weighing between 248 and 295 g, were used. They had been maintained in a room at $22 \pm 1^\circ\text{C}$ and were previously accustomed to being restrained in the rat stocks for several hours at a time. Each animal had freedom to move its limbs and neck and was in an alert condition. Between experiments, the animals were individually housed in wire-mesh cages with a 12-h light-dark cycle and fed with dry powder chow, with tap water available ad libitum.

Surgical preparation for drug injection or brain 5-HT assay

For the administration of drugs into the lateral cerebral ventricle, stainless-steel cannula, consisting of a guide tube (0.81-mm o.d.) with a snug fitting trocar, and a cannula insert, which was introduced into the tube just before injection, were used. The cannula guide tubes with trocars were implanted using coordinates found in the stereotaxic atlas of Paxinos and Watson (17). The animals were anesthetized with sodium pentobarbitone (6 mg/100 g, i.p.) and placed in a Kopf stereotaxic apparatus. After appropriately located craniotomy holes had been made, two self-tapping screws were attached to the calvarium of the parietal bones and the cannula guide tubes were inserted into the desired depth through the craniotomy holes. The cannula guide tubes were implanted stereotaxically into the lateral cerebral ventricle. They were anchored with fast-drying acrylic dental cement to the cranial surface which had been scraped clean of periosteum. The cannula insert was connected to a 10- μl Hamilton micro-syringe by P.E. 10 polyethylene tubing, and the syringe and tubing were filled with silicone fluid to act as a piston. The volume of injection into each cannula was 10 μl for intracerebroventricular (i.c.v.) injection. A period of 1 week was allowed to permit the animals to recover from the surgery before experimentation.

For measurement of 5-HT efflux in the hypothalamus, a 10-mm guide tube (21-gauge stainless-steel tubing) was implanted in the upper corner of the anterior commissure (A 8.7 mm from interaural line, L ± 0.8 mm, and V 6.7 mm below dura) (17). Following surgery, the guide cannula was plugged with a stylet and rats were returned to their home cages for a minimum recovery period of 1 week. The evening before an experiment, rats were briefly immobilized with a volatile anesthetic (Metofane; Pitman-Moore, Inc., Washington Crossing,

NJ, USA) and a dialysis probe was slowly lowered through the guide cannula and cemented in place. The dialysis probe was a CMA-12 microdialysis probe (2 mm in length) from Carnegie Medicine (CMA) (Stockholm, Sweden). The length of the dialysis probe was adjusted to place the probe tip at a point 0.5 mm above the base of the preoptic anterior hypothalamus (POAH: A 8.7 mm, L ± 0.8 mm, and V 8.7 mm below dura). The CMA-120 system (CMA) was used for microdialysis experiments in an awake, restrained animal. Ringer solution was used to perfuse microdialysis probes at 0.001 ml/min by a high precision pump (CMA-100). Sequential perfusates were collected by a microfraction collector (CMA-140) into polyethylene micro-sample tubes containing 0.005 ml of 0.1 M perchloric acid with 10 nl ascorbic (1 mg/ml) acid and 0.005 ml of the perfusates were analyzed for 5-HT on a microbore HPLC-ECD system. Performance of each microdialysis probe was calibrated by dialysis of a known amount of the standard mixture and recoveries of all analyses was then determined. Hypothalamic concentrations of 5-HT were calculated by determining each peak height ratio relative to the internal standard 3-methoxy tyramine (3-MT) and also corrected by each probe performance. Standard stock solution of 5-HT was prepared at the concentration of 0.02 ng/ml in 0.1 M perchloric acid and stored at -70°C in the dark and thawed in an ice bath prior to preparation of a standard mixture. The internal standard 3-MT and standard mixture were prepared fresh every day from a portion of the stock solutions after appropriate dilution with Ringer solution containing 10 nl ascorbic acid in 0.1 M perchloric acid.

In vitro tests were run to determine 5-HT recovery in the dialysis solution at the flow rate used. At room temperature, dialysis probes were immersed in dialysis solution containing 5-HT. At a flow rate of 1 $\mu\text{l}/\text{min}$ the relative (e.g., 5-HT concentration in the dialysis solution divided by 5-HT concentration in the beaker) was $12.6 \pm 1.4\%$ ($n = 12$). Basal 5-HT levels were considered stable after observation of 4 consecutive samples with no upward or downward trend. These basal values were averaged and all samples were expressed as a percent of the mean baseline. All probe placements were in the region of POAH as verified by standard histological procedures. Animals were perfused with physiological saline, followed by 10% formalin. The brains were removed, sliced on a freezing microtome, and stained with formyl thionine.

Drug solutions

All drug solutions were prepared using pyrogen-free glassware that was heated for 5 h at 180°C before use.

All drug solutions were prepared in pyrogen-free sterile 0.9% NaCl solution and passed through 0.22- μ m Swinnex bacterial filters. Drugs administered intraperitoneally (i.p.) included puerarin (1–30 mg/kg; Sigma Chemical Co., St. Louis, MO, USA), ketanserin (0.1–1.0 mg/kg; RBI, Natick, MA, USA), crude extracts of *Pueraria lobata* (self-made), and DOI (0.25–1.0 mg/kg, RBI). Drugs administered subcutaneously (s.c.) included 8-OH-DPAT (0.01–0.25 mg/kg, Sigma) and (–)-pindolol (RBI). Drugs administered i.c.v. included puerarin (50–100 μ g), 5,7-DHT (200 μ g, Sigma), and interleukin (IL)-1 β (10 ng, Sigma). All drug doses are expressed as the respective free base.

Preparation of crude extracts of *Pueraria lobata*

The dry root of *Pueraria lobata* was ground into a fine powder, and then the powder was added 10 vol methanol through a Soxhlet's apparatus at 60°C with continuous stirring. The solution was evaporated in a vacuum evaporator at 60°C, and the dry product was redissolved in distilled water. The water solutions of *Pueraria lobata* were used for thermal experiments.

Thermal tests

Each animal was used once. Throughout the experiment, colonic temperature (Tco) was measured every minute with a copper-constantan thermocouple connected to a thermometer (HP 1300; Yokogawa, Tokyo). The basal levels of Tco for each animal were allowed to stabilize for at least 90 min before any injection.

Microinjection was made into the third cerebral ventricle through an injecting cannula. The total volume of fluid (10 μ l) was injected over about 10 s. The effect of i.p. or i.c.v. injection of drugs on Tco were assessed in un-anesthetized rats at an ambient temperature of 24°C.

Data presentation and statistical analyses

Results are expressed as the mean $\pm$ S.E.M. Analysis of variance (ANOVA) was used for the factorial experiment, whereas Newman-Keuls' test was used for *post hoc* multiple comparisons among means. A *P* value less than 0.05 was considered to indicate a statistically significant difference.

Results

Figure 1 and Table 1 show the effects of i.p. administration of puerarin (1–30 mg/kg) or crude extracts of *Pueraria lobata* on Tco in normal rats. It can be seen from the figure and the table that systemic administration of these drugs produce a fall in Tco in rats. The hypothermia induced by systemic administration of puerarin or crude extracts of *Pueraria lobata* can be mimicked by i.c.v. injection of a lesser amount (50–100 μ g) of puerarin (Fig. 2). In addition, it was found that the hypothermia induced by puerarin or crude extracts of *Pueraria lobata* was associated with a reduction of 5-HT efflux in the rat hypothalamus (as shown in both Tables 1 and 2).

Both Tables 1 and 2 also demonstrate that pretreat-

Fig. 1. Time course of the effects of puerarin on colonic temperature (Tco) in rats. Puerarin (1, 5, 10, and 30 mg/kg) was injected intraperitoneally (i.p.) at time 0 min. The values are means $\pm$ S.E.M. of 12 rats per group. **P* < 0.05 and ***P* < 0.01, significantly different from the corresponding control value (vehicle group) (ANOVA followed by Newman-Keuls' test).

Table 1. Effects of intraperitoneal administration of puerarin or crude extracts of *Pueraria lobata* on both the colonic temperature (Tco) and the hypothalamic 5-HT concentrations in vehicle-pretreated and 5,7-dihydroxytryptamine (5,7-DHT)-pretreated rats

Treatments	ΔT_{co} (°C)	Hypothalamic 5-HT (% baseline)
Vehicle-pretreated rats		
Vehicle solution (ml/kg)	0.14 ± 0.05	99 ± 24
Puerarin, 1 mg/kg	0.23 ± 0.09	98 ± 22
Puerarin, 10 mg/kg	$-0.78 \pm 0.21^*$	$52 \pm 25^*$
Puerarin, 30 mg/kg	$-1.05 \pm 0.22^*$	$45 \pm 11^*$
<i>Pueraria lobata</i> , 2 g/kg	$-0.82 \pm 0.19^*$	$47 \pm 20^*$
5,7-DHT-pretreated rats		
Vehicle solution (ml/kg)	0.12 ± 0.04	$32 \pm 5^\dagger$
Puerarine, 1 mg/kg	0.13 ± 0.05	$29 \pm 4^\dagger$
Puerarine, 10 mg/kg	$-0.21 \pm 0.11^\dagger$	$27 \pm 3^\dagger$
Puerarine, 30 mg/kg	$-0.23 \pm 0.13^\dagger$	$22 \pm 2^\dagger$
<i>Pueraria lobata</i> , 2 g/kg	$-0.19 \pm 0.12^\dagger$	$23 \pm 2^\dagger$

The values are means $\pm$ S.E.M. of 12 rats per group. Δ , denotes the difference between the control value before and 120 min after the start of puerarin injection. The vehicle-pretreated control value for extracellular 5-HT release in the hypothalamus is 1.58 ± 0.43 pg/18 μ l per 30 min. $^*P < 0.05$, significantly different from the vehicle solution control value (two-way ANOVA followed by Newman-Keuls' test). $^\dagger P < 0.05$, significantly different from the vehicle-pretreated group (two-way ANOVA followed by Newman-Keuls' test). The Tco before treatment with drugs were within a normal range of 36–37°C in all groups of rats.

Fig. 2. Time course of the effects of puerarin on colonic temperature (Tco) in rats. Puerarin (50 or 100 μ g/10 μ l) was injected intracerebroventricularly (i.c.v.) at time 0 min. $^*P < 0.05$, significantly different from the corresponding control value (vehicle group) (ANOVA followed by Newman-Keuls' test).

ment with an i.c.v. dose of 200 μ g of 5,7-DHT (2 weeks before puerarin injection) not only reduces the hypothalamic 5-HT efflux but attenuates the hypothermia induced by puerarin or crude extracts of *Pueraria lobata*. In addition, the hypothermia induced by i.p. injection of puerarin can be attenuated by systemic injection of either (–)-pindolol, or DOI, but augmented by systemic administration of 8-OH-DPAT or ketanserin 90–120 min before puerarin injection. Systemic admin-

istration of (–)-pindolol or DOI alone causes hyperthermia, while systemic administration of 8-OH-DPAT or ketanserin alone causes hypothermia (Table 3). Serotonin depletion with 5,7-DHT did not affect basal body temperature.

Table 4 summarizes the effects of i.p. administration of puerarin 180 min after the pyrogen injection on both the febrile response and hypothalamic concentration of 5-HT response to i.c.v. injection of IL-1 β in rats. I.c.v.

Table 2. Effects of intracerebroventricular (i.c.v.) administration of puerarin on both the colonic temperature (Tco) and the hypothalamic 5-HT concentrations in vehicle-pretreated and 5,7-dihydroxytryptamine (5,7-DHT)-pretreated rats

Treatments	ΔT_{co} (°C)	Hypothalamic 5-HT (% baseline)
Vehicle-pretreated rats		
Vehicle solution (10 μ l), i.c.v.	0.12 ± 0.04	101 ± 18
Puerarin, 50 μ g (10 μ l), i.c.v.	0.24 ± 0.19	99 ± 17
Puerarin, 100 μ g (10 μ l), i.c.v.	$-0.95 \pm 0.24^*$	$41 \pm 4^*$
Puerarin, 150 μ g (10 μ l), i.c.v.	$-1.46 \pm 0.27^*$	$37 \pm 3^*$
5,7-DHT-pretreated rats		
Vehicle solution (10 μ l), i.c.v.	0.13 ± 0.05	30 ± 6
Puerarin, 50 μ g (10 μ l), i.c.v.	0.19 ± 0.12	28 ± 5
Puerarin, 100 μ g (10 μ l), i.c.v.	$-0.12 \pm 0.09^\dagger$	$25 \pm 3^\dagger$
Puerarin, 150 μ g (10 μ l), i.c.v.	$-0.64 \pm 0.22^\dagger$	$22 \pm 4^\dagger$

The values are means $\pm$ S.E.M. of 12 rats per group. Δ , denotes the difference between the control value before and 120 min after the start of puerarin injection. The vehicle-pretreated control value for extracellular 5-HT release in the hypothalamus is 1.58 ± 0.43 pg/18 μ l per 30 min. * $P < 0.05$, significantly different from the vehicle solution control values (two-way ANOVA followed by Newman-Keuls' test). $^\dagger P < 0.05$, significantly different from the vehicle-pretreated group (two-way ANOVA followed by Newman-Keuls' test). The Tco before treatment with drugs were within a normal range of 36–37°C in all groups of rats.

Table 3. Effects of systemic administration of (–)-pindolol, 8-OH-DPAT, ketanserin, or DOI 90–120 min before the puerarin injection on the thermal response to intraperitoneal (i.p.) injection of puerarin in rats

Treatments	ΔT_{co} (°C)
Vehicle (ml/kg, i.p.) + puerarin, 30 mg/kg (i.p.)	-1.11 ± 0.08
(–)-pindolol, 0.05 mg (s.c.)	0.42 ± 0.04
(–)-pindolol, 0.05 mg (s.c.) + puerarin, 30 mg/kg (i.p.)	$-0.63 \pm 0.08^*$
(–)-pindolol, 0.2 mg (s.c.)	0.65 ± 0.06
(–)-pindolol, 0.2 mg (s.c.) + puerarin, 30 mg/kg (i.p.)	$-0.46 \pm 0.06^*$
(–)-pindolol, 0.8 mg (s.c.)	0.83 ± 0.07
(–)-pindolol, 0.8 mg/kg (s.c.) + puerarin, 30 mg/kg (i.p.)	$-0.25 \pm 0.07^*$
8-OH-DPAT, 0.01 mg/kg (s.c.)	-0.34 ± 0.07
8-OH-DPAT, 0.01 mg/kg (s.c.) + puerarin, 30 mg/kg (i.p.)	-1.21 ± 0.07
8-OH-DPAT, 0.05 mg/kg (s.c.)	-0.64 ± 0.04
8-OH-DPAT, 0.05 mg/kg (s.c.) + puerarin, 30 mg/kg (i.p.)	$-1.56 \pm 0.11^*$
8-OH-DPAT, 0.25 mg/kg (s.c.)	-0.73 ± 0.06
8-OH-DPAT, 0.25 mg/kg (s.c.) + puerarin, 30 mg/kg (i.p.)	$-1.78 \pm 0.14^*$
Ketanserin, 0.1 mg/kg (i.p.)	-0.23 ± 0.05
Ketanserin, 0.1 mg/kg (i.p.) + puerarin, 30 mg/kg (i.p.)	-1.18 ± 0.09
Ketanserin, 0.5 mg/kg (i.p.)	-0.54 ± 0.06
Ketanserin, 0.5 mg/kg (i.p.) + puerarin, 30 mg/kg (i.p.)	$-1.45 \pm 0.10^*$
Ketanserin, 1.0 mg/kg (i.p.)	-0.65 ± 0.07
Ketanserin, 1.0 mg/kg (i.p.) + puerarin, 30 mg/kg (i.p.)	$-1.75 \pm 0.15^*$
DOI, 0.25 mg/kg (i.p.)	0.23 ± 0.04
DOI, 0.25 mg/kg (i.p.) + puerarin, 30 mg/kg (i.p.)	-1.07 ± 0.08
DOI, 0.5 mg/kg (i.p.)	0.75 ± 0.08
DOI, 0.5 mg/kg (i.p.) + puerarin, 30 mg/kg (i.p.)	$-0.33 \pm 0.05^*$
DOI, 1.0 mg/kg (i.p.)	1.14 ± 0.07
DOI, 1.0 mg/kg (i.p.) + puerarin, 30 mg/kg (i.p.)	$0.05 \pm 0.04^*$

The values are means $\pm$ S.E.M. of 12 rats per group. *Significantly different from the corresponding control value (vehicle + puerarin group) ($P < 0.05$, two way analysis of variance followed by Newman-Keuls' test). s.c., subcutaneous injection.

Table 4. Effects of intraperitoneal (i.p.) administration of puerarin 180 min after the pyrogen injection on both the febrile and hypothalamic concentration of 5-HT response to intracerebroventricular (i.c.v.) injection of IL-1 β in rats

Treatments	ΔT_{co} ($^{\circ}C$)	5-HT concentration (% baseline)
Vehicle (ml/kg, i.p.) + vehicle (10 μ l, i.c.v.)	0.14 $\pm$ 0.07	100 $\pm$ 24
Vehicle (ml/kg, i.p.) + IL-1 β (10 ng/10 μ l, i.c.v.)	1.98 $\pm$ 0.24*	386 $\pm$ 43*
Puerarin (1 mg/kg, i.p.) + IL-1 β (10 ng/10 μ l, i.c.v.)	1.74 $\pm$ 0.19	375 $\pm$ 38
Puerarin (10 mg/kg, i.p.) + IL-1 β (10 ng/10 μ l, i.c.v.)	0.86 $\pm$ 0.16 [†]	246 $\pm$ 29 [†]
Puerarin (30 mg/kg, i.p.) + IL-1 β (10 ng/10 μ l, i.c.v.)	0.58 $\pm$ 0.17 [†]	204 $\pm$ 14 [†]

The values are means $\pm$ S.E.M. of 10 rats per group. *Significantly different from the corresponding control values (vehicle + vehicle group) ($P < 0.05$, ANOVA followed by Newman-Keuls' test). [†]Significantly different from the corresponding control values (vehicle + IL-1 β group) ($P < 0.05$, ANOVA followed by Student-Newman-Keuls' test).

injection of IL-1 β causes an increase in both T_{co} and hypothalamic 5-HT release. The increased levels of both T_{co} and hypothalamic 5-HT efflux that occurred after IL-1 β injection are significantly attenuated by systemic injection of puerarin dose-dependently.

Discussion

The present results demonstrate that the serotonergic mechanisms in the hypothalamus are associated with the puerarin-induced hypothermia. We found that systemic administration of puerarin, in addition to producing hypothermia, produced a fall in 5-HT concentration in the hypothalamus. The hypothermia can be mimicked by direct injection of a lesser amount of puerarin into the cerebral ventricular system. The puerarin-induced hypothermia and decreased efflux of hypothalamic 5-HT were attenuated by selective depletion of 5-HT in the hypothalamus produced by intracerebral injection of 5,7-DHT, although both our present and previous (10) results showed that the 5-HT-depleted animals (after 5,7-DHT treatment) maintained normal body temperature. Furthermore, the puerarin-induced hypothermia was almost completely abolished by treatment with a 5-HT_{2A}-receptor agonist (e.g., DOI) or a 5-HT_{1A}-receptor antagonist (e.g., (-)-pindolol). In contrast, a 5-HT_{1A}-receptor agonist (e.g., 8-OH-DPAT) or a 5-HT_{2A}-receptor antagonist (e.g., ketanserin) had additive effects with puerarin. These results suggest that puerarin may act through 5-HT_{1A}-receptor activation and/or 5-HT_{2A}-receptor antagonism to exert its hypothermic effects. In fact, (-)-pindolol or DOI alone induces hyperthermia, while 8-OH-DPAT or ketanserin alone induces hypothermia. Our present results tend to show an additive relationship between puerarin and the above serotonergic agents, although there is no data showing the binding of puerarin to any serotonin receptors. The contention is supported by several previous results. An increase in both colonic temperature and hypothalamic

5-HT efflux of rats occurred following electrical stimulation of raphe nuclei (18). Local perfusion of the hypothalamus with dialysis solution containing 5-hydroxytryptophan (a 5-HT precursor), fluoxetine (a 5-HT-reuptake inhibitor), or high potassium significantly increased both colonic temperature and the extracellular concentrations of 5-HT in the hypothalamus (19). Systemic administration of 5-HT_{2A}-receptor agonists such as DOI, MK-212, or quipazine cause hyperthermia (15, 16, 20, 21). The hyperthermia induced by 5-HT_{2A}-receptor agonists was antagonized by treatment with 5-HT_{2A}-receptor antagonists such as ketanserin, LY 53857, mianserin, or ritanserin or with a 5-HT_{1A}-receptor antagonist such as spiperone. Reciprocally, both colonic temperature and extracellular concentration of 5-HT in the hypothalamus decreased with a dialysis solution containing tetrodotoxin, zero calcium concentration, or systemic administration of 8-OH-DPAT (a 5-HT_{1A}-receptor agonist) (19). The hypothermia and decreased 5-HT efflux in brain produced by systemic administration of 8-OH-DPAT can be reversed by blockade of 5-HT_{1A} receptors with (-)-pindolol (20, 22).

It should be mentioned that the hypothermic effects of puerarin, administered i.c.v., appeared more slowly and lasted longer than those of systemic route injection (10–20 mg), as shown in the present results. We suspect that diffusion barrier selectively retard the entrance from the cerebrospinal fluid into the postulated brain site of action. In addition, we suspect that extracellular catabolic enzymes in the peripheral blood stream would destroy the puerarin more effectively than those in the cerebrospinal fluid. Puerarin may act through dopaminergic, adrenergic, or other pathways to induce hypothermic effects. However, when the systemic dose was increased to 30 mg, the effects of puerarin were also as long as 6 h.

The hypothermic effects induced by 8-OH-DPAT were enhanced, rather than reduced, by pretreatment with 5,7-DHT or pCPA (20). The 8-OH-DPAT-induced

hypothermia has been attributed to activation of post-synaptic 5-HT_{1A} receptors (23, 24). On the other hand, our present results suggest that puerarin acts through the presynaptic 5-HT_{1A}-receptor activation to induce hypothermic effects, although there are no data showing that puerarin binds to any serotonin receptors. In addition, it should be stressed that puerarin shares with the crude extracts of *Pueraria lobata* the same thermal effects. This indicates that puerarin is the main constituent of *Pueraria lobata* in inducing hypothermia.

Other lines of evidence have also shown that activation of central adrenoceptors induces hyperthermic effects. For example, microinjection of norepinephrine into the cerebral ventricles of rabbit brain produced hyperthermic effects (25, 26). An increase in body temperature also resulted from an increase in the endogenous norepinephrine within the central nervous system immediately after administration of 6-hydroxydopamine (27) and desipramine (28). As stated in the introduction, puerarin has beta-adrenoceptor antagonism. Thus, it is likely that puerarin, in addition to acting through serotonergic receptor mechanisms, induces hypothermic effects by inhibiting adrenoceptor mechanisms in the brain.

It is generally believed that the release of lipopolysaccharide from Gram-negative bacterial cell wall during most infections can cause a fever by stimulating peripheral monocytes to synthesize and release several pyrogenic cytokines such as IL-1 β , tumor necrosis factor- α , and IL-6 (29). These pyrogenic cytokines enter the circulation and reach the endothelial cells of the hypothalamus. Prostaglandin E₂ (PGE₂) is released into the brain from the endothelial cells of the hypothalamus and binds to EP₃ receptors on the cells in the hypothalamic thermoregulatory center. This is followed by PGE₂-induced neuronal mechanisms to elevate the temperature set-point located in the hypothalamus, resulting in fever. Our unpublished results demonstrated that i.c.v. injection of either lipopolysaccharide, IL-1 β , or PGE₂, in addition to producing fever, stimulated the tryptophan hydroxylase expression in the hypothalamus. The present results further showed that i.c.v. injection of IL-1 β caused an increase in both colonic temperature and hypothalamic 5-HT efflux in rats. The IL-1 β -induced fever and increased hypothalamic 5-HT efflux could be attenuated by treatment with puerarin (present results) or 5-HT_{2A}-receptor antagonists such as cyproheptadine, ketanserin, or mianserin (unpublished data). The aforementioned statements tend to indicate that 5-HT_{1A}- and 5-HT_{2A}-receptor mechanisms in the hypothalamus are in dynamic balance in thermoregulation. Accordingly, activation of 5-HT_{1A} and 5-HT_{2A} receptors in the hypothalamus induces hypothermic and hyper-

thermic effects, respectively. IL-1 β may induce fever by activating 5-HT_{2A} receptors and/or antagonizing 5-HT_{1A} receptors in the hypothalamus. The systemic or central administration of puerarin may activate 5-HT_{1A} receptors and/or antagonize 5-HT_{2A} receptors in the hypothalamus to exert its hypothermic and/or antipyretic effects. Effect of 1 mg/kg puerarin under IL-1 β was not significantly different from IL-1 β alone (Table 4), so hypothermic effects and antipyretic effects can not be distinguishable.

Acknowledgments

This work was supported by grants from the National Science Council (NSC 90-2316-B-010-009) and the Ministry of Education (89-B-FA22-1-4-02) of the Republic of China.

References

- 1 Lu XR, Gao E, Xu LZ, Li HZ, Kang B, Chen WN, et al. Blocking effect of puerarin on beta-adrenoceptors of isolated organs and the whole animal. *Acta Pharmacol Sin.* 1986;7:537–539. (text in Chinese with English abstract)
- 2 Wang LY, Zhao AP, Chai XS. Effects of puerarin on rat vascular smooth muscle in vitro. *Acta Pharmacol Sin.* 1994; 15:180–182. (text in Chinese with English abstract)
- 3 Overstreet DH, Lee YW, Rezvani AH, Pei YH, Criswell HE, Janowsky DS. Suppression of alcohol intake after administration of the Chinese herbal medicine, NPI-028, and its derivatives. *Alcohol Clin Exp Res.* 1996;20:221–227.
- 4 Fan LL, Sun LH, Li J, Yue XH, Yu HX, Wang SY. The protective effect of puerarin against myocardial reperfusion injury. Study on cardiac function. *Chin Med J.* 1992;105:11–17.
- 5 Song XP, Chen PP, Chai XS. Effects of puerarin on blood pressure and plasma renin activity in spontaneously hypertensive rats. *Acta Pharmacol Sin.* 1988;9:55–58. (text in Chinese with English abstract)
- 6 Chai XS, Wang ZX, Chen PP, Wang LY, Lu XR, Kang B. [Anti-arrhythmic action of puerarin]. *Acta Pharmacol Sin.* 1985;6: 166–168. (text in Chinese with English abstract)
- 7 Zhou Y, Su X, Cheng B, Jiang J, Chen H. [Comparative study on pharmacological effects of various species of *Pueraria*]. *Zhongguo Zhong Yao Za Zhi.* 1995;20:619–621, 640. (in Chinese)
- 8 Lin MT, Chuang JI. Melatonin potentiates 5-HT_{1A} receptor activation in rat hypothalamus and results in hypothermia. *J Pineal Res.* 2002;33:14–19.
- 9 Matsuda T, Yoshikawa T, Suzuki M, Asano S, Somboonthum P, Takuma K, et al. Novel benzodioxan derivative, 5-(3-(((2S)-1,4-benzodioxan-2-ylmethyl) amino) propoxy)-1,3-benzodioxole HCl (MKC-242), with a highly potent and selective agonist activity at rat central serotonin 1A receptors. *Jpn J Pharmacol.* 1995;69:357–366.
- 10 Lin MT. Effects of brain monoamine depletions on thermoregulation in rabbits. *Am J Physiol.* 1980;238:R364–R371.
- 11 Frankfurt M, Mendelsen SD, Mckittrick CR, McEwen BS.

- Alterations of serotonin receptor binding in the hypothalamus following acute denervation. *Brain Res.* 1993;601:349–352.
- 12 Hjorth S, Carlsson A, Lindberg P, Sanchez D, Wikstrom H, Arvidsson L-E, et al. 8-Hydroxy-2-(di-n-propylamino) tetralin, 8-OH-DPAT, a potent and selective simplified ergot congener with central 5-HT-receptor stimulating activity. *J Neural Transm.* 1982;55:169–188.
- 13 Middlemiss DN, Fozard JR. 8-Hydroxy-2-(di-n-propylamino)-tetralin discriminates between subtypes of the 5-HT₁ recognition site. *Eur J Pharmacol.* 1983;90:151–153.
- 14 Hjorth S. Hypothermia in the rat induced by the potent serotonergic agent 8-OH-DPAT. *J Neural Transm.* 1985;61:131–135.
- 15 Nash JF, Meltzer HY, Gudelsky GA. Antagonism of serotonin receptor mediated neuroendocrine and temperature responses by atypical neuroleptics in the rat. *Eur J Pharmacol.* 1988;151:463–469.
- 16 Yamawaki S, Lai H, Horita A. Dopaminergic and serotonergic mechanisms of thermoregulation: mediation of thermal effects of apomorphine and dopamine. *J Pharmacol Exp Ther.* 1983;227:383–388.
- 17 Paxinos G, Watson C. The rat brain in stereotaxic coordinates. New York: Academic Press; 1982.
- 18 Sheard MH, Aghajanian GK. Neural release of brain serotonin and body temperature. *Nature.* 1967;216:495–496.
- 19 Lin MT, Tsay HJ, Su WH, Chueh FY. Changes in extracellular serotonin in rat hypothalamus affect thermoregulatory function. *Am J Physiol.* 1998;274:R1260–R1267.
- 20 Hutson PH, Donohoe TP, Curzon G. Hypothermia induced by the putative 5-HT_{1A} agonist LY165163 and 8-OH-DPAT is not prevented by 5-HT depletion. *Eur J Pharmacol.* 1987;143:221–228.
- 21 Salmi P, Karlsson T, Ahlenius S. Antagonism by SCH 23390 of clozapine-induced hypothermia in the rat. *Eur J Pharmacol.* 1994;253:67–73.
- 22 Goodwin GM, De Souza RJ, Green AR. The pharmacology of the hypothermic response in mice to 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT). A model of presynaptic 5-HT₁ function. *Neuropharmacology.* 1985;24:1187–1194.
- 23 Martin KF, Phillips I, Hearson M, Prow MR, Heal DJ. Characterization of 8-OH-DPAT-induced hypothermia in mice as a 5-HT_{1A} autoreceptor response and its evaluation as a model to selectively identify antidepressants. *Br J Pharmacol.* 1992;107:15–21.
- 24 Millan MJ, Rivet JM, Canton H, Marouille-Girardon S, Gobert A. Induction of hypothermia as a model of 5-hydroxytryptamine_{1A} receptor-mediated activity in the rat: a pharmacological characterization of the actions of novel agonists and antagonists. *J Pharmacol Exp Ther.* 1993;264:1364–1376.
- 25 Cooper KE, Cranston WI, Honour AJ. Effects of intraventricular and intrahypothalamic injection of noradrenaline and 5-HT on body temperature in conscious rabbits. *J Physiol (Lond).* 1965;181:852–864.
- 26 Tangri KK, Bhargava AK, Bhargava KP. Interrelation between monoaminergic and cholinergic mechanisms in the hypothalamic thermoregulatory centre of rabbits. *Neuropharmacology.* 1974;13:333–346.
- 27 Lewis PJ, Rawlins MD, Reid JL. Acute thermoregulatory and cardiovascular effects of 6-hydroxydopamine administered centrally in rabbits and cats. *Br J Pharmacol.* 1972;46:559.
- 28 Cranston WI, Hellon RF, Luff RH, Rawlins MD. Hypothalamic endogenous noradrenaline and thermoregulation in the cat and rabbit. *J Physiol (Lond).* 1972;223:59–67.
- 29 Dinarello CA, Gatti S, Bartfai T. Fever: links with an ancient receptor. *Curr Biol.* 1999;9:R147–R150.