Catecholaminergic Mechanisms-Mediated Hypothermia Induced by Magnolol in Rats

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ABSTRACT—Intraperitoneal administration of magnolol (25–100 mg/kg) produced a dose-related fall in rats' colonic temperature. The magnolol-induced hypothermia was attenuated by pretreatment with intracerebroventricular 6-hydroxydopamine (200 μ g/rat). The L-DOPA (200 mg/kg, i.p.) plus benserazide (50 mg/kg, i.p.)-induced hyperthermia was attenuated by magnolol. On the other hand, the α -methyltyrosine (100 mg/kg, i.p.)-induced hypothermia was potentiated by magnolol. Furthermore, magnolol (50 mg/kg, i.p.) decreased the dopamine and norepinephrine release in the hypothalamus, but did not change the concentrations for their metabolites (3,4-dihydroxyphenylacetic acid and homovanillic acid). The data suggest that magnolol decreases colonic temperature by reducing catecholaminergic activity in rat hypothalamus.

Keywords: Magnolol, Hypothermia, Catecholamine

The bark of *Magnolia officinalis* (Houpo) has been prescribed as a Chinese medicine for the relief of anxiety, stroke, headache and fever. Magnolol, a biphenyl compound, was the main constituent isolated from the bark of *Magnolia officinalis* (1). It has a variety of pharmacological activities, including central nervous inhibition (2), anti-anxiety (3), inhibition of platelet aggregation and thromboxane formation (4), and anti-inflammatory and analgesic effects (5). To our knowledge, little information is available about the hypothermia induced by magnolol. Therefore in the present study, the effects of magnolol on colonic temperature was investigated. Hypothalamic catecholamines are believed to be involved in the thermoregulation of the body (6). Furthermore, the present study would also provide information about the role of hypothalamic catecholamines in magnolol-induced hypothermia.

Male Sprague-Dawley rats (250–300 g) were used in the present study. Upon receipt from the supplier (Animal Resource Center, China Medical College, Taichung, Taiwan), the animals were housed in a temperature-regulated ($22 \pm 1^\circ\text{C}$) room that was kept on a 12/12-hr light/dark cycle. Experiments were performed during the light phase of the cycle. Animals were provided with food and water ad libitum. They were previously trained to keep quiet under minimal restraint in a

special rat stock for periods of several hours at a time (7). The animal had freedom to move his limbs and neck as well as being in an alert condition.

Temperature was measured in a temperature-controlled room (ambient temperature $22 \pm 1^\circ\text{C}$). The colonic temperature was measured every minute with a copper-constantan thermocouple connected to a thermometer (HR1300; Yokogawa, Tokyo). The copper-constantan thermocouple was enclosed in polyethylene 200 tubing, sealed at one end, and inserted 60 mm into the colon. The colonic temperature of each animal was allowed to stabilize for at least 60 min before any injections. Basal values are those taken immediately before the injection (time 0) of magnolol, L-DOPA, α -methyltyrosine (α -MT) or vehicle alone. α -MT and magnolol are injected simultaneously. L-DOPA was injected 20 min before magnolol. Benserazide was injected 10 min after magnolol.

The rats, under sodium pentobarbital anesthesia, received a single injection of 6-hydroxydopamine (6-OHDA, 200 μ g in 5 μ l), dissolved in 0.9% saline containing 0.1% ascorbic acid, into the lateral cerebral ventricle. The control rats were treated with an intracerebroventricular dose of 5 μ l 0.9% saline containing 0.1% ascorbic acid. Seven days after 6-OHDA or vehicle injection, the animals were used for the experiment (8).

For surgery, rats were anesthetized with sodium pen-

tobarbital (45 mg/kg, i.p.) and placed in a Kopf stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA) in the flat skull position. A 10-mm guide tube cannula (21-gauge stainless-steel tubing) was planted in the upper corner of the anterior commissure (A 8.7 mm, L $\pm$ 0.8 mm and V 6.7 mm below the dura) (9). Following surgery, the guide cannula was plugged with a stylet. The rats were returned to their cages for a minimum recovery period of 1 week.

Before the experiment, a dialysis probe (CMA-12; Carnegie-Medicine, Stockholm, Sweden) was slowly lowered through the guide cannula and cemented in place. The rats were restrained in rat stocks. Ringer's solution was perfused through the microdialysis probe at a rate of 1 μ l/min by a high precision pump (CMA-100, Carnegie-Medicine) attached to a liquid swivel. Sequential 20- μ l aliquots of the dialysis solution were collected by a microfraction collector (CMA-140, Carnegie-Medicine) into polyethylene microsample tubes; 5 μ l of the dialysis solution was analyzed for catecholamines, including dopamine (DA), epinephrine (Epi) and norepinephrine (NE), and their metabolites, including 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), on a microbore high-performance liquid chromatography with an electrochemical detector system (HPLC-ECD; PM-60 pump/LC-4C ECD; Bio Analytical System, West Lafayette, IN, USA). The column was a microbore reverse phase column (GSK-C18; 5- μ m ODS, 1.0 $\times$ 150 mm, I.D.; Bio Analytical System).

The mobile phase composition was 50 ml acetonitrile, 9.45 g monochloroacetic acid, 186 mg ethylenediamine-tetra-acetic acid and 160 mg sodium 1-octanesulfonate in doubled distilled water. The solution was adjusted to pH 3.0 by concentrated orthophosphoric acid, and its final volume was adjusted to 1.0 l. The mixture was filtered with a 0.22- μ m nylon filter under reduced pressures and degassed for 20 min (10).

Magnolol was isolated and purified from the cortex of *Magnolia officinalis*, purity of almost 95%, and was dissolved in 0.5% carboxy methyl cellulose (CMC)-saline (2). The other drugs were obtained from Sigma Chemicals (St. Louis, MO, USA). 6-OHDA was dissolved in 0.9% saline containing 0.1% ascorbic acid. L-DOPA, benserazide and α -MT were dissolved in 0.5% CMC-saline.

Basal catecholamine levels and thermoregulatory parameters were considered stable after observation of 60 min with no upward or downward trend. The catecholamine levels of all samples were expressed as a percent of the baseline. Student's *t*-test was used when only two groups were compared. ANOVA was used for factorial experiments, while Student's *t*-test was used for post hoc multiple comparisons among means. The criterion for statistical significance was set at $P < 0.05$.

Table 1. Effects of magnolol on the colonic temperature in normal or 6-OHDA-pretreated rats

Treatment (mg/kg, i.p.)	Changes in colonic temperature (Δ °C)	
	Vehicle-treated rats	6-OHDA-treated rats
Control	-0.16 $\pm$ 0.05	0.11 $\pm$ 0.10
Magnolol 25	-0.98 $\pm$ 0.08*	-0.13 $\pm$ 0.14
Magnolol 50	-1.42 $\pm$ 0.14**	-0.16 $\pm$ 0.22
Magnolol 100	-2.34 $\pm$ 0.33***	-0.22 $\pm$ 0.17

The values are the mean $\pm$ S.E.M. from 8 rats for each group. Δ , difference between the control values before and 60 min after the injection. The control value for vehicle and 6-OHDA-treated rats are 37.8 $\pm$ 0.12°C and 38.2 $\pm$ 0.14°C, respectively. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, significantly different from the corresponding control group.

Table 1 summarizes the effects of magnolol on normal body temperature in rats with or without 6-OHDA pretreatment. In normal rats, magnolol (25, 50, 100 mg/kg, i.p.) produced a dose-related fall in colonic temperature at room temperature (22°C). The magnolol-induced hypothermia was attenuated by pretreatment with intraventricular 6-OHDA (200 μ g).

Table 2 shows that intraperitoneal administration of L-DOPA (200 mg/kg, i.p.) plus benserazide (50 mg/kg, i.p.) increased basal level of colonic temperature. Its hyperthermia was attenuated by magnolol. On the other hand, the α -MT (100 mg/kg, i.p.) decreased the basal level of colonic temperature. Its hypothermia was potentiated by magnolol.

Figure 1 shows the time course of the effects of magnolol on colonic temperature and extracellular dopamine, epinephrine, norepinephrine and their metabolites (DOPAC and HVA) in the hypothalamus of conscious rats. In vivo microdialysis data revealed that magnolol (50 mg/kg, i.p.) produced a decrease of dopamine and norepinephrine concentrations in the hypothalamic dialysates, but did not change the concentrations of their metabolites.

Brodie and Shore first postulated that the hypothalamus plays an important role in the regulation of body temperature (11). The evidence suggests that catecholamine pathways play a modulatory role in the maintenance of thermoregulation (6). 6-OHDA, a specific neurotoxin for catecholaminergic neurons, when injected into the cerebral ventricular system primarily causes the degeneration of catecholamine-containing axons and axonal terminals (12). Zoeren and Stricker reported that pretreatment of rats with 6-OHDA in the hypothalamus produces no significant alterations in rectal temperature (13). As show in the Table 1, we obtained the similar results. The hypothermic effects of magnolol were at-

Table 2. Effects of L-DOPA and α -methyltyrosine (α -MT) on the hypothermic response of magnolol in rat's colonic temperature

Treatment (mg/kg, i.p.)	Changes in colonic temperature ($\Delta^{\circ}\text{C}$)		
	Vehicle	L-DOPA + Benserazide	α -MT
Control	-0.15 ± 0.04	0.64 ± 0.19	-0.79 ± 0.20
Magnolol 25	$-0.95 \pm 0.21^*$	$-0.88 \pm 0.22^*$	$-1.68 \pm 0.23^*$
Magnolol 50	$-1.46 \pm 0.25^{**}$	$-1.40 \pm 0.31^{**}$	$-2.11 \pm 0.37^{**}$

Values are means $\pm$ S.E.M. of 8 rats for each group. Δ , difference between the control values before and 60 min after the start of magnolol injection. L-DOPA (200 mg/kg) was injected 20 min before the magnolol injection. Benserazide (50 mg/kg) was injected 10 min after the magnolol injection. α -MT (100 mg/kg) and magnolol are injected simultaneously. $^*P < 0.05$, $^{**}P < 0.01$, significantly different from the corresponding control group, ANOVA.

Fig. 1. Time course of the effects of magnolol on colonic temperature and extracellular DA, NE, Epi, DOPAC and HVA release in the hypothalamus of conscious rats. Intraperitoneal injection of magnolol at 50 mg/kg (●) or vehicle (○) was performed at time 0 min. The values are the mean $\pm$ S.E.M. of 4 animals. The vehicle-treated control values for extracellular DA, NE, Epi, DOPAC and HVA release in the hypothalamus are 7.7 ± 1.2 , 35.5 ± 1.5 , 1.1 ± 0.5 , 2.8 ± 1.0 and 3.7 ± 0.9 ng/20 μ l/20 min, respectively. $^aP < 0.05$, $^bP < 0.01$ or $^cP < 0.001$, significantly different from the corresponding vehicle-treated control values, Student's *t*-test.

tenuated in rats with 6-OHDA pretreatment. These results indicated that the magnolol-induced hypothermia might be due to the decrease in the presynaptic activity of catecholaminergic neurons.

The present results demonstrate that intraperitoneal injection of magnolol elicited a proportional decrease in the colonic temperature, which could be mimicked by intraperitoneal injection of tyrosine hydroxylase inhibitors such as α -MT (14). Tyrosine is the amino acid precursor of dopamine, epinephrine and norepinephrine. The rate-limiting step in the biosynthesis of these catecholamines is the hydroxylation of tyrosine to form DOPA by tyrosine hydroxylase. Several tyrosine analogs, such as α -MT, can inhibit tyrosine hydroxylase (14). On the other hand, DOPA is decarboxylated to form dopamine by DOPA decarboxylase. The hydroxylation of dopamine at the beta carbon to form norepinephrine is mediated by the enzyme dopamine β -hydroxylase (14). Lin reported that intracerebroventricular administration of L-DOPA produced hyperthermia (due to cutaneous vasoconstriction) at both 22°C and 30°C ambient temperature (15). The magnolol-induced hypothermia was potentiated by treatment with α -MT. Intraperitoneal administration of L-DOPA plus benserazide (DOPA decarboxylase inhibitor) slightly increased basal levels of colonic temperature. The hyperthermia induced by L-DOPA plus benserazide was attenuated by magnolol. From the microdialysis experiment, magnolol decreased the levels of dopamine and norepinephrine in hypothalamic dialysates at the same time. These results suggest that magnolol decreases colonic temperature by reducing catecholaminergic activity in the rat's hypothalamus.

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