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# Enhancement of Vasoconstrictor Responses to 5-HT but no to Methoxamine by Cooling in Isolated Dog Lingual and Mesenteric Arteries

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Tsukada, M. & Chiba, S. *Enhancement of Vasoconstrictor Responses to 5-HT but no to Methoxamine by Cooling in Isolated Dog Lingual and Mesenteric Arteries*. Tohoku J. Exp. Med., 2000, **191** (3), 139–144 —— The effect of temperature on submaximal vasoconstrictions to an intraluminal injection of serotonin (5-HT) and methoxamine was investigated in isolated and perfused canine lingual and mesenteric arteries, using the cannula insertion method. In both arteries cooling (from 37°C to 27°C) caused a remarkable enhancement of vasoconstriction to 5-HT, but did not to methoxamine. In lingual arteries, methoxamine-induced constrictions were strongly depressed, although those were slightly depressed in mesenteric arteries. It is assumed that 5-HT produces an important role to modulate vascular tonicity in low temperature conditions. ———— cannula insertion method; canine lingual artery; mesenteric artery; cooling; 5-HT 

Since the body temperature is greatly regulated by the cutaneous blood flow, temperature changes influence the blood vessel reactivity as reviewed by Vanhoutte (1980). As the circulation is controlled by the sympathetic vasoactive system, the effect of temperature on adrenergic responses of arterial vasculature has been reported in isolated vessel preparations (Vanhoutte and Lorenz 1970a; Peiper et al. 1971; Patton and Wallace 1978; Flavahan et al. 1985). It is well known that cutaneous arteries and veins are equipped with a mixed population of postjunctional $\alpha_1$- and $\alpha_2$-adrenoceptors (De Mey and Vanhoutte 1981; Steen et al. 1984; Ito and Chiba 1985; Lindblad and Ekenvall 1986). The dog tongue has been recognized physiologically as the regulatory organ for the body temperature (Pleschka 1984). Previously it is demonstrated that there are abundant functional $\alpha_1$- but no $\alpha_2$-adrenoceptors in isolated canine lingual and mesenteric arteries (Chiba and Tsukada 1984; Skrbic and Chiba 1991). In the present study we made an attempt to investigate effects of temperature change (from 37°C to

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27°C) on vasoconstrictor responses to an $\alpha_1$-adrenoceptor agonist methoxamine and a potent vasoactive substance serotonin (5-HT), since 5-HT is an endogenous amine existing in platelets and is readily released into circulation.

## Materials and Methods

Twelve mongrel dogs (6–14 kg) of either sex were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). The animals were killed by rapid exsanguination from the right common carotid artery after treatment with sodium heparin (200 units/kg, i.v.). The method was previously reported (Hongo and Chiba 1983; Tsuji and Chiba 1984). Briefly, the lingual and superior mesenteric arteries were carefully isolated and dissected into several segments (11 mm in length, $1.3 \pm 0.1$ mm outer diameter in the lingual artery [$n=12$], and $1.1 \pm 0.1$ mm in the mesenteric artery [$n=12$]). Side branches were tied with thread in the arteries. In both arteries, surrounding connective tissues were carefully removed. Each segment was cannulated with a stainless-steel cannula (25 gauge and 3 cm in length) which had 3 small holes at a distance of 3 mm from the distal blind end. The arterial segment was fixed to the cannula by a thin thread distal from the holes. Thus, the stream of perfusate, after passing the holes of the cannula, circulated only through the intraluminal surface of the arterial segment. The cannulated preparations were set up in the organ bath and perfused with Krebs-Henseleit solution at constant flow (1.6–1.8 ml/min) by means of a microtube pump (MP-3A, Tokyorika, Tokyo). The perfusate contained (mM): NaCl 118; KCl 4.7; $CaCl_2$ 2.5; $MgSO_4$ 1.2; $KH_2PO_4$ 1.2; $NaHCO_3$ 25 and glucose 11, bubbled with a mixture of 95% $O_2$ and 5% $CO_2$ to maintain the pH level at 7.4. The entire system was maintained at a constant temperature of 37°C (control) by means of a thermostat pump (Haake, Dieselster, Germany). When the temperature changed from 37°C to 27°C or from 27°C to 37°C, it took approximately 10–15 minutes in every case. Perfusion pressure was monitored by a pressure transducer (TP-400T, Nihon Kohden, Tokyo) coupled to a recording system (WT-685GH, Nihon Kohden). The temperature of perfusing Krebs solution was increased or decreased by adjusting the temperature of the reservoir, using the thermostat pump (Haake, Dieselster). The organ bath and Krebs-Henseleit solution temperatures were monitored by a thermistor probe suspended in the organ bath and perfusing solution. The drug (serotonium creatinine sulfate, 5-HT or methoxamine hydrochloride) was given by a single bolus injection in volume of 10 or 30 $\mu$l and the injection time was approximately 1 or 3 seconds, respectively, by use of a microsyringe (MS-50, Ito Co., Shizuoka). Individual drugs were repeatedly administered at a constant dose and interval. The administered dose was chosen by approximate 50% of effective concentration ($EC_{50}$). To avoid tachyphylaxis, an administration of each substance was performed at adequate intervals over 7 minutes for methoxamine and 10 minutes for 5-HT, respectively. After equilibration, the preparations were subjected to repeated

test concentrations of drugs until reproducible constrictions developed. Responses to drugs were not significantly altered for a period of over 2 hours after constant responses observed. The responses were calculated as percentage of the mean responses to drug at 37°C (control) in each artery. Results are expressed as the means±S.E.M.. Comparisons between the mean response in the control period and the responses in the subsequent experimental periods were made using a one-way ANOVA followed by Bonferroni's test. A $p$-value less than 0.05 was considered to be significant.

## Results

Following a 60 minutes equilibration period, the basal perfusion pressure was 44±2 mmHg ($n=10$) on lingual arteries and 44±4 mmHg ($n=12$) on superior mesenteric arteries at 37°C, and a fall of 10°C from the control value of 37°C (cooling) increased the basal perfusion pressure on both arteries (57±2 mmHg, lingual arteries [$n=10$]; 51±4 mmHg, superior mesenteric arteries [$n=12$]). When the bath temperature was restored to the control level of 37°C (rewarming), the perfusion pressure was not significantly different to the basal control perfusion pressure. The changes in temperature in a range of 37 to 27°C did not affect the pH level in the perfusing solution. 5-HT (0.27±0.04 nmol, lingual arteries [$n=10$]; 0.21±0.02 nmol, superior mesenteric arteries [$n=12$]) increased the per-

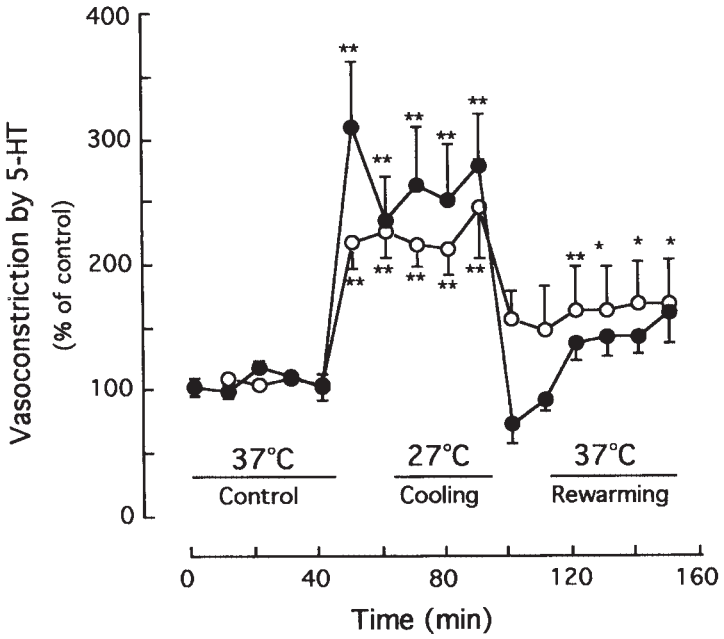


Fig. 1. Influences of cooling (from 37°C to 27°C) and rewarming (from 27°C to 37°C) on 5HT-induced vasoconstrictions in isolated and perfused canine lingual and mesenteric arteries. Doses of 5-HT were 0.27±0.04 nmol ($n=10$) in lingual arteries, and 0.21±0.02 nmol ($n=12$) in mesenteric arteries, respectively. Data are the mean±S.E.M.. $^{*}p<0.05$, $^{**}p<0.01$ compared with control.
○, Lingual ($n=7$-10); ●, Mesenteric ($n=7$-10).

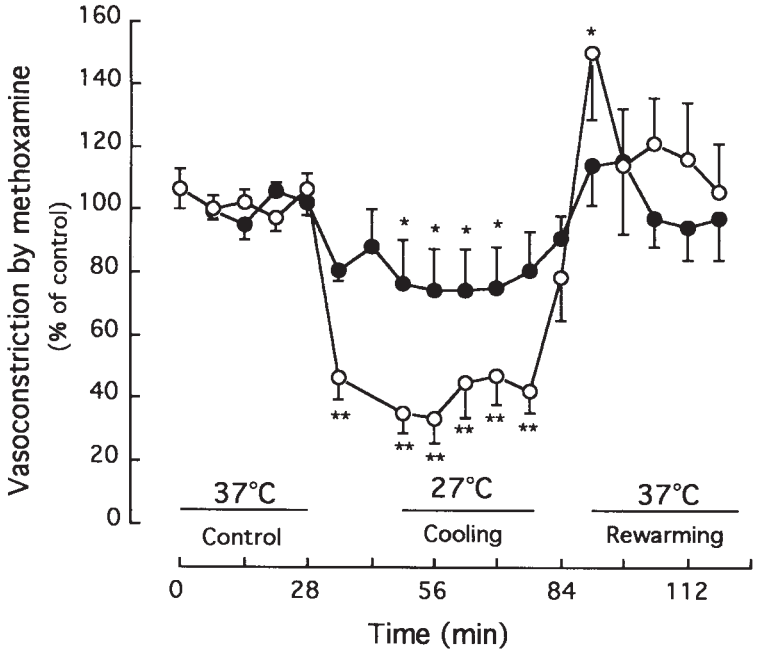


Fig. 2. Influences of cooling (from 37°C to 27°C) and rewarming (from 27°C to 37°C) on methoxamine-induced vasoconstrictions in isolated and perfused canine lingual and mesenteric arteries. Doses of methoxamine were 43±14 nmol ($n=6$) in lingual arteries, and 24±4 nmol ($n=12$) in mesenteric arteries, respectively. Data are the mean±S.E.M.. $^{*}p<0.05$, $^{**}p<0.01$ compared with control.
○, Lingual ($n=4$–6); ●, Mesenteric ($n=8$–12).

fusion pressure to 70±13 mmHg and 50±8 mmHg, respectively at 37°C. Fig. 1 shows that 5-HT-induced vasoconstrictions were markedly potentiated during cooling (27°C) in either lingual or superior mesenteric arteries. After rewarming, 5-HT-induced responses returned to the responses of the control level (37°C) in mesenteric arteries. In lingual arteries the responses were still significantly potentiated after rewarming. On the other hand, the responses to methoxamine were not potentiated but depressed during cooling (27°C) especially in the lingual artery as shown in Fig. 2.

## Discussion

It has been reported that cooling to 20–29°C causes an increase of the constriction of cutaneous blood vessels induced by a variety of vasoactive substances (Vanhoutte 1980). In the canine saphenous veins, the potentiation by cooling of the response to noradrenaline may reflect an instantaneous increase in affinity of the postjunctional $\alpha$-adrenoceptor for noradrenaline (Janssens and Vanhoutte 1978). Since cutaneous vessels contains abundant postjunctional $\alpha_2$-adrenoceptors, the potentiation may be due to hypersensitivity of $\alpha_2$-adrenoceptors. However, in this study, since methoxamine has no $\alpha_2$-stimulating properties, such hypersensitivity may be not induced. During cooling the methoxamine-induced effects were rather depressed. As both kinds of arteries

used have predominant $\alpha_1$-adrenoceptors but no $\alpha_2$-receptors, it is considered that $\alpha_1$-adrenoceptors were depressed by cooling. On the contrary, 5-HT-induced responses were strongly potentiated during cooling in this study. In cutaneous veins and arteries of the dog, cooling potentiates contractions induced by 5-HT (Vanhoutte and Shepherd 1970b; Lindblad and Ekenvall 1986). Van Nueten et al. (1985) suggested that moderate cooling may increase the affinity of the 5-$HT_2$ receptors of cutaneous vascular smooth muscle. Harker et al. (1991) reported that acute moderate cooling (37 to 24°C) did not significantly augment KCl-induced constrictions of human saphenous veins or rat tail arteries but it enhanced 5-HT-induced constrictions.

In the present study, in the lingual artery we demonstrated that cooling enhances vasoconstrictions by 5-HT but depresses methoxamine-induced vasoconstrictions. The mechanism that in lingual arteries the methoxamine-induced action was strongly depressed more than in mesenteric arteries may be due to different $\alpha_1$-adrenoceptor subtypes. As the canine tongue has an important role to modulate the temperature in the body, the augmented vasoconstriction to 5-HT in the lingual artery may rapidly and strongly contribute to the prevention of the heat-loss. It seems likely that 5-HT is a most important vasoactive biogenic amine in low temperature conditions. The physiological role of 5-HT on peripheral vasculature tonicity in cold conditions is unclear, but it is not ruled out that 5-HT released from platelets regulate vascular reactivity to temperature changes.

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