Effect of Oral OPC-28326, a Selective Femoral Arterial Vasodilator, on Hindlimb Subcutaneous Tissue Temperature in Conscious Dogs Under Buprenorphine Sedation

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ABSTRACT—In dogs, rectal temperature was decreased to about 36°C at 2 to 2.5 h after sedation with buprenorphine (0.3 mg/kg, i.m.), and hindlimb subcutaneous tissue temperature (TSC) in the thigh decreased in a similar manner. TSC in the dorsum of the foot showed a greater decrease than that in the thigh. OPC-28326 (4-(N-methyl-2-phenylethylamino)-1-(3,5-dimethyl-4-propionyl-aminobenzoyl) piperidine hydrochloride monohydrate) at doses of 0.3, 1.0 and 3.0 mg/kg, p.o. inhibited the buprenorphine-induced decrease in TSC in the dorsum dose-dependently, but had little effect on that in the thigh or rectal temperature. In conclusion, TSC in the extremities were more sensitive to core temperature and peripheral circulation.

Keywords: Peripheral circulation, Subcutaneous temperature, Conscious dog

OPC-28326 (4-(N-methyl-2-phenylethylamino)-1-(3,5-dimethyl-4-propionyl-aminobenzoyl) piperidine hydrochloride monohydrate) is a newly developed, selective vasodilator increasing the blood flow of the femoral artery (1). This drug has little effect on other cardiovascular parameters, such as blood pressure, heart rate, contractile force of myocardium, and blood flow in other areas, such as the carotid, vertebral, coronary, renal and mesentery arteries (1). α2-Adrenoceptor blocking action of OPC-28326 may be involved its selective vasodilator action on the femoral artery (2). To evaluate the effects of oral administration of OPC-28326, we measured subcutaneous temperature using a wire-type thermometer in conscious dogs sedated with buprenorphine. It has been reported that intravenously injected buprenorphine exerted hypothermic action on body temperature in a chronic spinal dog (3). We found that buprenorphine decreased subcutaneous temperature in the periphery (the dorsum of the foot) more greatly than that in the thigh of dogs. In the present study, we examined the effects of OPC-28326 on subcutaneous temperature in the dogs sedated with buprenorphine, and we measured rectal temperature and subcutaneous temperature in the dorsum of the foot and thigh.

Male beagle dogs weighing 9 – 12 kg were housed during the experiment in a sound-proof room in which temperature and humidity were controlled at 23 ± 2°C and 60 ± 10%, respectively. A thermometer (Electronic thermometer; Seiwa ME Laboratory, Tokyo) was inserted into the rectum. After the animals were restrained in a standing position, buprenorphine at the dose of 0.3 mg/kg was administered intramuscularly. Changes in rectal temperature were measured for 5 h at 30-min intervals. The changes in rectal temperature of buprenorphine-treated dogs were compared with those of non-treated dogs.

Dogs were restrained in a standing position in slings, and buprenorphine was injected into the muscles of the left thigh. When the dogs were adequately sedated (approximately 30 min after buprenorphine), a needle-type probe (TN-800; Unique Medical, Tokyo) of a biothermometer (TME-300, Unique Medical) was placed in the subcutaneous tissues in the dorsum of the right foot and in the right thigh to measure subcutaneous temperature. Temperature was recorded on a thermal pen recorder (Recti-Horiz 8K; NEC Medical Systems, Tokyo). Subcutaneous tissue temperature (TSC) in the dorsum of the foot continueds to de-
crease after buprenorphine. OPC-28326 (0.3, 1 and 3 mg /kg) or its vehicle (distilled water, 1 ml/kg) was administered orally using a gastric tube when temperature fell to about 30°C.

OPC-28326 (Otsuka Pharmaceutical Co., Ltd., Tokyo) was dissolved in distilled water and administered in a volume of 0.3 ml/kg or 1 ml/kg using a gastric tube. The vehicle of OPC-28326 was administered in a volume of 1 ml/kg. Buprenorphine (0.2 mg/ml, Lepetan Injection®; Otsuka Pharmaceutical Co., Ltd.) was administered intramuscularly in a volume of 1.5 ml/body.

Values are expressed as the mean ± S.E.M. All of the analyses were performed by the Statistical Analysis System (SAS Institute Japan, Tokyo). The differences in subcutaneous temperature between the OPC-28326 groups and the vehicle group were analyzed by analysis of variance (ANOVA) based on repeated measurements. The differences at each time point were analyzed by the two-tailed Dunnett’s multiple comparison test. Differences were considered statistically significant at P<0.05.

The rectal temperature at the start of the experiment was 38.4 ± 0.1°C and 38.3 ± 0.2°C in the buprenorphine-treated and non-treated groups, respectively. Buprenorphine produced a decrease in rectal temperature, which reached a plateau after 2 h (Fig. 1A). In the non-treated group, rectal temperature was only slightly decreased (Fig. 1A).

The effects of buprenorphine on T_s in the dorsum of the foot and the thigh are shown in Fig. 1B. T_s in the dorsum of the foot and the thigh was 34.7 ± 0.4°C and 35.0 ± 0.3°C, respectively, at approximately 30 min after administration of buprenorphine (0.3 mg/body, i.m.). T_s in the thigh was decreased but little changed after 2–2.5 h. The decrease in T_s in the thigh paralleled that in body core temperature (cf., Fig. 1A). On the other hand, T_s in the dorsum of the foot decreased progressively (Fig. 1B).

![Fig. 1.](image1)

**Fig. 1.** Effects of intramuscular administration of buprenorphine (0.3 mg/body) on rectal temperature and subcutaneous tissue temperature. A: Time course changes in rectal temperature. Black circles indicate dogs with buprenorphine treatment and white circles indicate dogs without buprenorphine. Each point represents the mean ± S.E.M. of 3 animals. B: Time course changes in subcutaneous tissue temperature in the dorsum of the foot (black circles) and in the thigh (gray circles). Each point represents the mean ± S.E.M. of 10 animals.

![Fig. 2.](image2)

**Fig. 2.** Effects of oral administration of OPC-28326 on hindlimb subcutaneous tissue temperature in the dorsum of the foot (A) and in the thigh (B) in the dog with intramuscular buprenorphine administration (0.3 mg/body). In this experiment, control (distilled water, black circles), OPC-28326 at 0.3 mg/kg (white circles), OPC-28326 at 1.0 mg/kg (white triangles) or OPC-28326 at 3.0 mg/kg (white squares), was administered when the subcutaneous tissue temperature in the dorsum of the foot was around 30°C (cf., Fig. 1). Time-response curve of the control group was remade from that of Fig. 1. Time point at which the subcutaneous tissue temperature became around 30°C was set at 0 h. OPC-28326 or its vehicle was administered at 0 h. Each point represents the mean ± S.E.M. of 10 animals (control group) and 5 animals (OPC-28326 treated group). Inset: the statistical results of two-way ANOVA based on repeated measurement in the control and each dose of OPC-28326. *P<0.05, **P<0.01 compared with control (Dunnett’s multiple comparison test).
foot markedly decreased, continued to decrease even after \( T_{SC} \) in the thigh reached a constant level, up to 5 h after buprenorphine, and reached a plateau at 24.3 \( \pm \) 0.5°C.

When \( T_{SC} \) in the dorsum of the foot fell to approximately 30°C after buprenorphine, OPC-28326 or its vehicle was orally administered. In the vehicle group, \( T_{SC} \) in the dorsum of the foot continued to decrease as described above. At doses of 0.3 and 1 mg/kg, OPC-28326 inhibited the decrease in \( T_{SC} \) in a dose-dependent manner. The patterns of the \( T_{SC} \) decrease were significantly different from those of the vehicle group (Statistical interaction effect: \( P<0.01 \)). In the group of OPC-28326 at a dose of 3 mg/kg, \( T_{SC} \) decreased until 1.5 h after administration, then gradually increased, and reached almost pretreatment levels at 4 h compared to the control group (Fig. 2A).

There was a significant difference in \( T_{SC} \) in the thigh between the groups of OPC-28326 at a dose of 0.3 mg/kg and the vehicle from the viewpoint of statistical interaction effect. However, the degree was negligible, and a dose-response relation could not be obtained; there was no significant difference between the control and higher doses (1.0 and 3.0 mg/kg) of OPC-28326 (Fig. 2B). Thus, OPC-28326 has little biological effect on \( T_{SC} \) in the thigh. OPC-28326 did not affect rectal temperature at all (data not shown).

In the present study, we found that buprenorphine caused a decrease in rectal temperature. This result is consistent with a report of Martin et al. (3) that buprenorphine induced a dose-related decrease in body temperature when injected intravenously into a spinal dog. Since buprenorphine is designated as a \( \mu \)-opioid receptor partial agonist, it may have similar effects, including hypothermia, to morphine, but its hypothermic action is not as strong as that of morphine (3). Buprenorphine had no effect on blood pressure, heart rate or femoral blood flow, even when a higher dose (1 mg/kg) was injected intravenously into anesthetized dogs (4). Taken together, it is thought that buprenorphine induces hypothermic action through the activation of \( \mu \) opioid receptors without direct action on hemodynamics such as vasoconstriction in subcutaneous tissues.

\( T_{SC} \) in the thigh and in the dorsum of the foot decreased after buprenorphine injection, suggesting that a decrease in core temperature strongly affects peripheral subcutaneous temperature at the extremities. It is thought that peripheral temperature is determined by various factors, such as heat loss from body surface through radiation, convection, and transpiration and heat gain by conduction from deep tissues, metabolic thermogenesis, and skin blood flow. In the present experiment, dogs were sedated and allowed to rest in a temperature- and humidity-controlled room, and heat loss from the skin is probably stable. The decrease in \( T_{SC} \) in the thigh is parallel to that in body temperature. Thus, it appears that heat gain by conduction from deep tissues might decrease in this area. \( T_{SC} \) in the dorsum of the foot, on the other hand, was much lower and independent of the decrease in body temperature; the temperature continued to decrease for 5 h and reached 24.3 \( \pm \) 0.5°C, which was close to room temperature (23 \( \pm \) 2°C). It has been reported that vasoconstrictor tone was maintained at a high level in the foot but was low in the thigh (5), and blood flow in the hindpaw, largely in cutaneous tissues, decreased (6) when the animals were exposed to low temperature. These studies suggest that the decrease in \( T_{SC} \) in the dorsum of the foot is related to the decrease in heat supply. Johnson et al. (7) have reported that heat conductance in cutaneous tissues increases, when cutaneous blood vessels, such as capillary and arteriovenous anastomoses, dilate. Thus, cutaneous blood flow may be one of the important factors in the regulation of skin temperature.

OPC-28326 is a selective vasodilator of the hindlimb, increasing femoral blood flow with little effect on other cardiovascular functions (1). Oral administration of OPC-28326 increased \( T_{SC} \) in the thigh of a dog without affecting core temperature in this study. We have already showed that OPC-28326 did not have an affinity for opiate receptors (1). Therefore, it may be a reasonable explanation that OPC-28326 increases heat supply to the cutaneous area of the dorsum of the foot through increasing blood flow. We suggested that \( \alpha_2 \)-adrenoceptor antagonistic action may be involved in the mechanism of the vasodilator action of OPC-28326 (1). We also suggested that properties of the \( \alpha_2 \)-adrenoceptor antagonistic action of OPC-28326 showed high selectivity for postjunctions compared to yohimbine, and OPC-28326 did not affect the central nervous action induced by an \( \alpha_2 \)-adrenoceptor agonist (2).

OPC-28326 showed affinities for \( \alpha_{2A} \), \( \alpha_{2B} \) and \( \alpha_{2C} \)-adrenoceptors with \( K_i \) values of 2040, 285 and 55 nM, respectively, in receptor binding studies (2). It was reported that cooling augmented vasoconstriction induced by \( \alpha_{2C} \)-adrenoceptor activation and that postjunctional \( \alpha_2 \)-adrenoceptors are important for thermoregulation in this vascular bed (8, 9). More recently, it was demonstrated that vasoconstriction induced by the cold was augmented preferentially via \( \alpha_{2C} \)-adrenoceptors, which were silent in a warm environment (10). Taken together, the effects of OPC-28326 shown in this study might be responsible for its \( \alpha_{2C} \)-adrenoceptor-blocking action.

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