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**Effect of P2 receptor blockade on cutaneous vasodilation during rest
and exercise in the heat in young men**

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*P2 receptors and skin blood flow during exercise*31 **ABSTRACT**

32 We assessed the role of purinergic P2 receptors in the regulation of cutaneous vasodilation in
33 young adults at rest and during intermittent moderate-intensity exercise in the heat (35°C). P2
34 receptor blockade augmented resting cutaneous vasodilation but had no influence during and
35 following exercise. This increase was partly diminished by nitric oxide synthase inhibition.
36 These results suggest a functional role of P2 receptors in the regulation of cutaneous vascular
37 tone during ambient heat exposure at rest. (75/75 words)

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39 **Key words:** ATP, microcirculation, heat loss

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INTRODUCTION

P2 purinergic receptors, which are primarily activated by adenosine triphosphate (ATP), play diverse roles in the regulation of various physiological systems. However, the extent of their involvement in the regulation of cutaneous vasodilation and thereby the regulation of a stable body core temperature in humans, remains to be elucidated. Human skin is differentiated into glabrous (e.g., hand) and nonglabrous (e.g., forearm) skin, the latter of which covers a comparatively greater portion of the body surface and plays an important role in the exchange of heat. In normothermic resting humans, ATP administration induces nonglabrous cutaneous vasodilation (Wingo et al. 2010; Fujii et al. 2015; Kalsi et al. 2017). Moreover, passive heating of the limb has been shown to induce elevations in erythrocyte-derived ATP (Kalsi et al. 2017). It is therefore plausible that P2 receptor activation contributes to nonglabrous cutaneous vasodilation during heat (i.e., exposure to a hot environment and/or exercise) strain.

We recently reported that nitric oxide (NO) synthase (NOS) is involved in the cutaneous vasodilatory response to exogenous ATP under normothermic resting conditions (Fujii et al. 2015). NOS is a primary mediator of cutaneous vasodilation during exercise (Welch et al. 2009; Meade et al. 2015) and, therefore, P2 receptor activation may induce cutaneous vasodilation during exercise via NO-dependent mechanisms. In this study, we tested the hypothesis that P2 receptors contribute to the regulation of cutaneous vasodilation during intermittent exercise in the heat through NOS-dependent pathway.

MATERIALS AND METHODS

Ethical Approval

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This study was approved by the University of Ottawa Health Sciences and Science Research Ethics Board and conformed to the guidelines set out in the *Declaration of Helsinki*. Verbal and written informed consent was obtained from all volunteers prior to their participation.

Participants

Twelve young males participated in the current study. Participants' age, body mass, height, surface area, and peak oxygen uptake were (mean $\pm$ SD) 25 ± 5 years, 76.7 ± 5.4 kg, 1.76 ± 0.08 m, 1.94 ± 0.09 m², and 47.1 ± 9.1 ml·kg⁻¹·min⁻¹, respectively. Participants were healthy, physically active non-smokers and free of known respiratory, cardiovascular and metabolic diseases. They were also not taking prescription medication.

Experimental design

On separate days, participants completed one preliminary and one experimental session. Prior to attending both sessions, participants were instructed to refrain from taking over-the-counter medication, supplements (e.g., vitamins) and performing strenuous exercise for at least 24 h. Participants were also asked to refrain from caffeine for at least 12 h and to eat a light meal no less than 2 h prior to participation. During the preliminary session participants' physical characteristics were obtained (protocol detailed elsewhere (Meade et al. 2015)).

The experimental session was conducted in a temperature controlled chamber (Can-Trol Environmental Systems Limited, Markham, ON, Canada) regulated to 35°C and 20% relative humidity. Four microdialysis fibers (>4 cm apart between sites) implanted into the dermal layer of the forearm skin were continuously perfused at a rate of 4 μ l·min⁻¹ in a counter-balanced manner with either: 1) lactated Ringer's solution (**Control**), 2) 100 mM pyridoxine (**PDX**;

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Sigma-Aldrich, St. Louis, MO, USA), a non-selective P2 receptor blocker, 3) 10 mM N^G -nitro-L-arginine methyl ester (**L-NAME**; Sigma-Aldrich), a non-selective NOS inhibitor, or 4) a combination of 100 mM pyridoxine + 10 mM L-NAME (**PDX+L-NAME**). The concentration used for L-NAME was based on previous studies (Welch et al. 2009; Meade et al. 2015). Further, pyridoxine (vitamin B12) was used to inhibit P2 receptor activity via the metabolically active form of pyridoxal-5-phosphate. The concentration used for pyridoxine was determined during pilot work wherein we identified 100 mM as the lowest concentration required to maximally attenuate cutaneous vasodilation induced by 30 mM ATP administration. Pyridoxine and L-NAME were perfused for a minimum of 60 min prior to data collection to ensure the establishment of each blockade and administration of each agent was continued throughout the trial (until the maximal skin blood flow protocol).

Following a 10-min baseline resting period, participants performed two consecutive 30-min bouts of cycling at a fixed rate of metabolic heat production of 400 W. Both bouts of exercise were followed by 20 min recovery periods.

Measurements

Forearm cutaneous red blood cell flux was locally measured at a sampling rate of 32 Hz with laser-Doppler flowmetry (PeriFlux System 5000, Perimed, Stockholm, Sweden) and integrated laser Doppler flowmetry probe (Model 413, Perimed). The laser-Doppler probe was placed directly over the centre of each microdialysis membrane. Systolic and diastolic blood pressures were obtained every 5 min throughout the experimental protocol using manual auscultation with a mercury column sphygmomanometer (Baumanometer Standby Model, WA Baum Co, Copiague, NY, USA). Cutaneous vascular conductance (CVC) was calculated as cutaneous red

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blood cell flux divided by mean arterial pressure and presented as a percentage of maximal CVC (%CVC_{max}). Maximal CVC as defined by a stable plateau in CVC maintained for ≥ 2 min (obtained at the end of the experiment) was achieved by the administration of 50 mM sodium nitroprusside (Sigma-Aldrich) at a rate of 6 $\mu\text{l}\cdot\text{min}^{-1}$. Heart rate was recorded using a Polar coded WearLink and transmitter, Polar S400 interface and Polar Trainer 5 software (Polar Electro, Kempele, Finland). Esophageal temperature was measured using a paediatric thermocouple probe (Mon-a-therm, Mallinckrodt Medical, St. Louis, MO, USA). Skin temperature was measured at 4 skin sites and mean skin temperature was calculated using the following regional distribution: chest, 30%; biceps, 30%; quadriceps, 20%; and calf, 20%. Metabolic energy expenditure was estimated using electrochemical gas analyzers (AMETEK model S-3A/1 and CD3A, Applied Electrochemistry, Pittsburgh, PA, USA).

Data analysis

Baseline resting CVC was obtained by averaging measurements performed over the last 5 min of the 10-min baseline period. CVC representing the end of exercise was obtained by averaging the last 5 min of each exercise bout. CVC obtained at the end of the first and second recovery was averaged from min 10 to 15 of the 20 min recovery periods. Difference in CVC between the Control and PDX site was evaluated as P2 receptor dependent change in cutaneous vascular tone. Also, difference in CVC between the L-NAME and PDX+L-NAME sites was evaluated as P2 receptor dependent change in cutaneous vascular tone without the influence of NOS during baseline resting. Esophageal and mean skin temperatures, as well as heart rate are presented as the average of data collected over the last 5 min of each time period. Blood pressure is presented as averages of the two last measurements made at the end of each time period.

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133 **Statistical analysis**

134 CVC was analyzed using a two-way repeated-measures analysis of variance with the factors of
135 treatment site and time. Body temperatures and cardiovascular responses were analyzed using a
136 one-way repeated-measures analysis of variance with the factors of time. CVC_{max} obtained
137 during sodium nitroprusside perfusion was assessed with a one-way repeated-measures analysis
138 of variance with the factors of treatment site. When a main effect was observed, *post hoc*
139 analysis was performed using two-tailed Student's paired or independent samples t-tests, where
140 appropriate, adjusted for multiple comparisons using the Hochberg's procedure, a modified
141 version of Bonferonni test. One-tailed Student's dependent samples t-test was employed to
142 compare the difference in CVC between the Control and PDX site versus that between the
143 L-NAME and PDX+L-NAME site during baseline resting. For all analyses, the level of
144 significance was set at $P \leq 0.05$. Statistical analyses were completed using the software package
145 SPSS 24.0 for Windows (IBM, Armonk, NY). Values are reported as means $\pm$ 95% confidence
146 interval, unless otherwise noted.

147

148 **RESULTS**

149 CVC was augmented relative to the Control site at the PDX site during baseline resting ($P \leq$
150 0.05), but not during either exercise bout ($P > 0.05$) or recovery period ($P > 0.05$) (Figure 1).
151 Further, CVC was reduced compared to Control at the L-NAME ($P \leq 0.05$) and PDX+L-NAME
152 ($P \leq 0.05$) sites throughout the experimental protocol (except PDX+L-NAME site during
153 recovery 2, $P > 0.05$). At the PDX+L-NAME site, CVC was higher than at the L-NAME site
154 during baseline resting and recovery 1 ($P \leq 0.05$). During baseline, the difference in CVC

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between the Control and PDX sites was greater than between the L-NAME and PDX+L-NAME sites (18 ± 8 vs. 9 ± 5 %max, $P \leq 0.05$). CVC_{max} did not differ between treatment sites ($P > 0.05$).

Esophageal and mean skin temperatures and heart rate were elevated during and following both exercise bouts in comparison to baseline (Table 1, $P \leq 0.05$).

DISCUSSION

We showed that CVC during rest in the heat was augmented with PDX, suggesting that an endogenous increase in ATP during exposure to heat at rest leads to cutaneous vasoconstriction via P2 receptor activation. Our observations however are in contrast to previous findings of cutaneous vasodilation during intradermal administration of ATP in young adults under normothermic resting conditions (Wingo et al. 2010; Fujii et al. 2015). It should be highlighted however, that a recent study by Lang et al. (2017) demonstrated cutaneous vasoconstriction in resting normothermic humans during low-dose ATP administration. It is therefore plausible that ATP can either cause cutaneous vasodilation or vasoconstriction; a response which is likely dependent on the dose of ATP and/or ambient temperature conditions.

In general, increased interstitial ATP can cause activation of vascular smooth muscle P2 receptors (Burnstock and Ralevic 2014) thereby inducing vasoconstriction. Although not quantified in the current study, increased interstitial ATP may be achieved via release from sympathetic nerves secondary to elevated sympathetic activation (Burnstock and Kennedy 1986). In line with this, increased skin and sympathetic nerve activity has been observed during whole-body heat stress (Niimi et al. 1997; Kamijo et al. 2011). Alternatively, elevated blood temperature (which would occur with increased body core temperature during exercise) can

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increase intravascular ATP released from erythrocytes (Kalsi et al. 2017), which may move to the interstitial space, activating vascular smooth muscle cells P2 receptors.

During baseline resting, the elevation in cutaneous vasodilation elicited by PDX was diminished when NOS was inhibited simultaneously ($9 \pm 5\%CVC_{\max}$ difference between PDX and Control) compared to when it was not ($18 \pm 8\%CVC_{\max}$ difference between PDX+L-NAME and L-NAME). This may indicate that the P2 receptor dependent reduction in cutaneous perfusion observed in the current study is partly NOS-dependent, though future work is required to elucidate the underpinning mechanisms. Alternatively, the P2 receptor-mediated decrease in CVC may be associated with lower CVC during NOS inhibition.

We showed that NOS contributes to cutaneous vasodilation during exercise in the heat (Figure 1) in accordance with previous reports (Welch et al. 2009; McNamara et al. 2014; Meade et al. 2015). However, we did not observe a role for P2 receptors in the modulation of cutaneous vasodilation during exercise in the heat. Similarly, our results showed that P2 receptors do not modulate cutaneous vasodilation postexercise. The absence of a P2 receptor modulation of cutaneous vasodilation during and following exercise may be the result of factors associated with performing exercise in the heat such as those associated with an elevated body temperature beyond that observed during rest. These factors may override or modulate the extent to which P2 receptors cause cutaneous vasoconstriction. For example, it is plausible that an exercise-induced increase in body core temperature may be associated with a concomitant increase in intravascular ATP concentration. This possibility is supported by the findings of Kalsi et al. (2017) who showed ATP release from erythrocytes increases in a temperature-dependent manner. Higher doses of ATP are associated with marked cutaneous

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vasodilation (Wingo et al. 2010), which could counteract the cutaneous vasoconstrictor effect thereby resulting in no measurable change in cutaneous vascular tone during exercise in the heat.

It should be noted that we evaluated exercise responses at a fixed metabolic heat production of 400 W, which is equivalent to a light-to-moderate exercise intensity. Had we employed a higher exercise intensity, and therefore higher metabolic heat load, body core temperature would have increased to a greater extent. Consequentially, this would have likely been associated with greater ATP production given that ATP increases in a temperature-dependent manner as discussed above (Kalsi et al. 2017). In that case, a P2 receptor-dependent modulation of cutaneous vascular tone might be evident, as intensity dependent differences in the mechanisms of cutaneous vasodilation is possible. Specifically, a recent report demonstrated that the contribution of NOS to cutaneous vasodilation is diminished during exercise at higher rates of metabolic heat production (Meade et al. 2015).

Conclusion

We showed that P2 receptors do not modulate cutaneous vasodilation during intermittent exercise in the heat. However, P2 receptors attenuate cutaneous vasodilation during baseline rest in the heat; a response that may occur through NOS-dependent mechanisms.

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DISCLOSURES

No conflict of interest, financial or otherwise, are declared by the author(s). The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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*P2 receptors and skin blood flow during exercise*274 **Table 1.** Body temperatures and cardiovascular responses

	Baseline	Exercise 1	Recovery 1	Exercise 2	Recovery 2
	10 min	30 min	20 min	30 min	20 min
Esophageal temperature (°C)					
(n = 11)	36.99 ± 0.11	37.44 ± 0.12†	37.24 ± 0.10†	37.57 ± 0.12†‡	37.36 ± 0.11†‡
Mean skin temperature (°C)					
(n = 9)	34.78 ± 0.43	35.53 ± 0.20†	35.30 ± 0.22†	35.58 ± 0.19†	35.34 ± 0.22†
Mean arterial pressure (mmHg)					
(n = 12)	90 ± 2	96 ± 5†	88 ± 4	95 ± 5	87 ± 2
Heart rate (beats·min⁻¹)					
(n = 12)	70 ± 6	113 ± 7†	79 ± 7†	117 ± 8†‡	79 ± 7†

275 Values presented as means ± 95% confidence interval. †P ≤ 0.05 vs. Baseline. ‡P ≤ 0.05 Exercise 1 vs. Exercise 2 or Recovery 1 vs.

276 Recovery 2.

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FIGURE LEGENDS

Figure 1. Time course changes in forearm cutaneous vascular conductance during intermittent exercise performed at a fixed rate of metabolic heat production of 400 W. Four forearm skin sites were continuously perfused via intradermal microdialysis with: 1) lactated Ringer’s solution (Control); 2) 100 mM pyridoxine (PDX), a non-selective P2 receptor inhibitor; 3) 10 mM NG-nitro-L-arginine methyl ester (L-NAME), a non-selective NOS inhibitor; or 4) a combination of 100 mM PDX + 10 mM L-NAME (PDX+L-NAME). Values are mean ± 95% confidence interval. Ex 1, first exercise; Rec 1, first recovery; Ex 2, second exercise; and Rec 2, second recovery. † vs. Control site; ‡ L-NAME vs. PDX + L-NAME (all $P \leq 0.05$).

Figure 1