Heat loss responses to intradermal administration of ATP

Lyra Halili, Naoto Fujii, Ryan McGinn, Maya Sarah Singh, Narihiko Kondo and Glen P. Kenny
Human and Environmental Physiology Research Unit, School of Human Kinetics, University of Ottawa, Canada

Introduction

Adenosine triphosphate (ATP) may potentially be involved in the control of cutaneous active vasodilation and sweating during heat stress, both of which are crucial in maintaining stable body temperature in humans.

Previous studies have demonstrated mechanisms of cutaneous active vasodilation and sweating during passive heating at rest and during exercise in the heat, however the exact pathways remain equivocal. Whether ATP can modulate thermoeffector organs such as skin vessels and eccrine sweat glands is unknown.

Cutaneous vasodilation in humans *in vivo* can be induced via intradermal administration of ATP. Previous studies with ACh have shown a combined role of nitric oxide synthase (NOS) and cyclooxygenase (COX) in modulating cutaneous active vasodilation.

Moreover, purinergic receptors are present in human sweat glands and human skin. ATP has been shown to increase sweating *in vitro*, however, sweating responses differ *in vivo*. Further, ATP catabolizes to adenosine in humans and adenosine when bound to P1 purinergic receptors can influence cutaneous vascular regulation. Both pathway type (NOS and/or COX) and receptor type (P1 or P2) in which ATP modulates cutaneous vasodilation *in vivo* is unknown.

Methodology

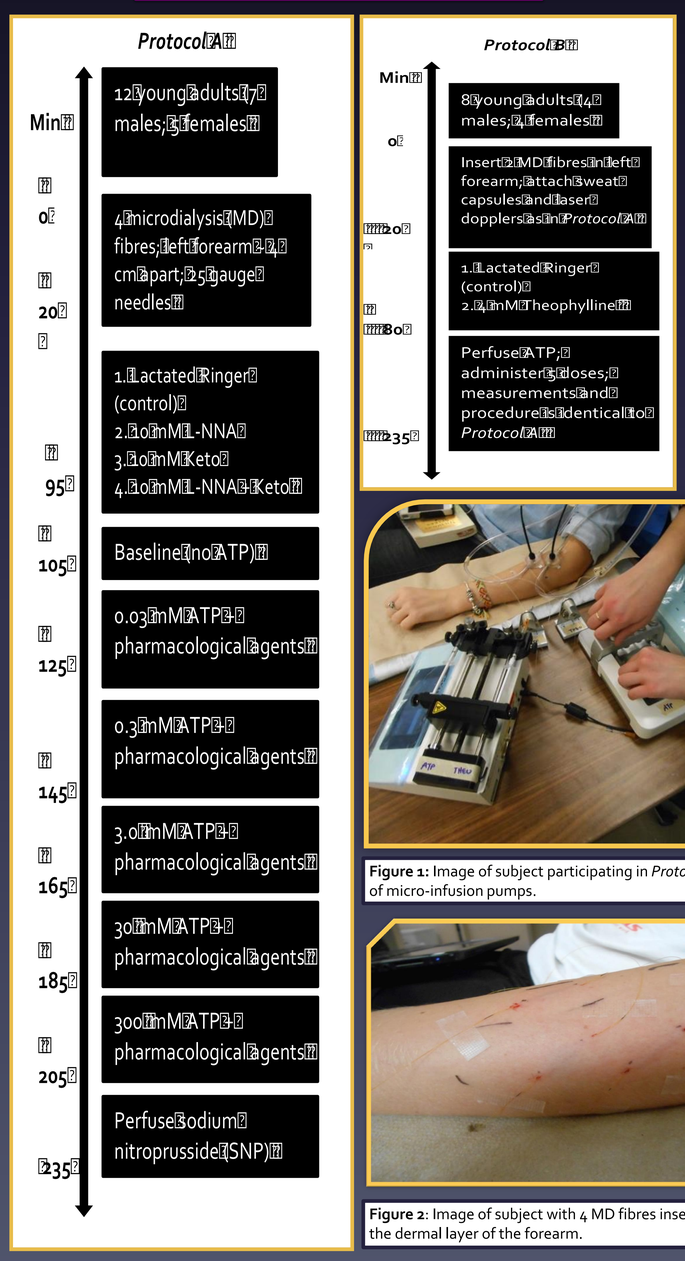
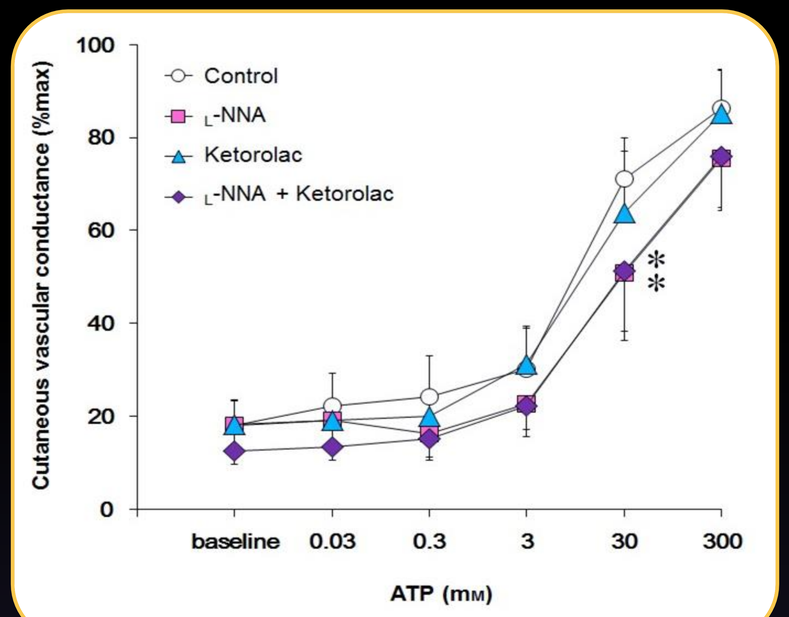


Figure 1: Image of subject participating in *Protocol 2* and set up of micro-infusion pumps.

Figure 2: Image of subject with 4 MD fibres inserted into the dermal layer of the forearm.

Results

a)



b)

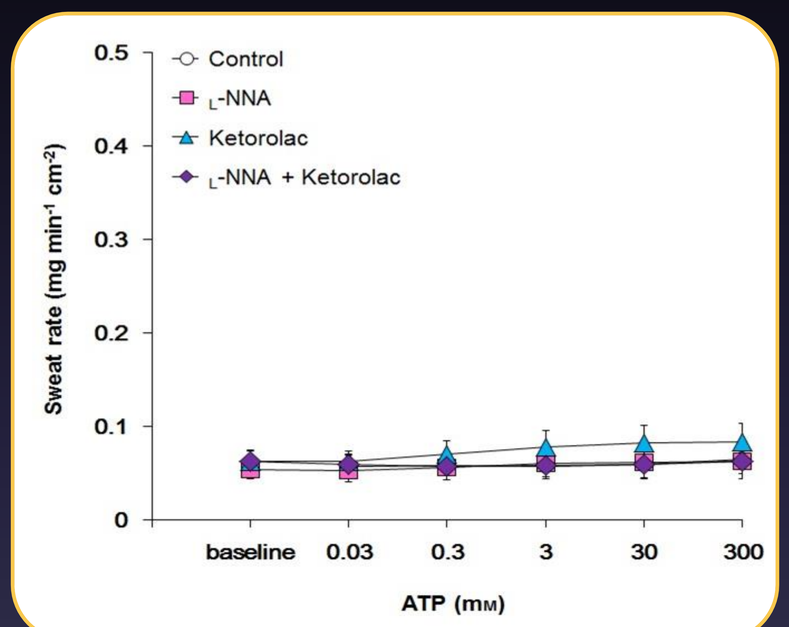


Figure 3: a) Cutaneous vascular conductance at baseline and during ATP administration from 0.03 to 300 m_M at 4 skin sites. **b)** Sweat rate at baseline and during ATP administration from 0.03 to 300 m_M at four skin sites. Sites: 1) lactated Ringer (**Control**), 2) 10 m_M N^G-nitro-$_L$-arginine ($_L$**-NNA**- non-selective NOS inhibitor), 3) 10 m_M ketorolac (**Ketorolac**-non-selective COX inhibitor) or 4) combination of 10 m_M $_L$-NNA and ketorolac ($_L$**-NNA + Ketorolac**)

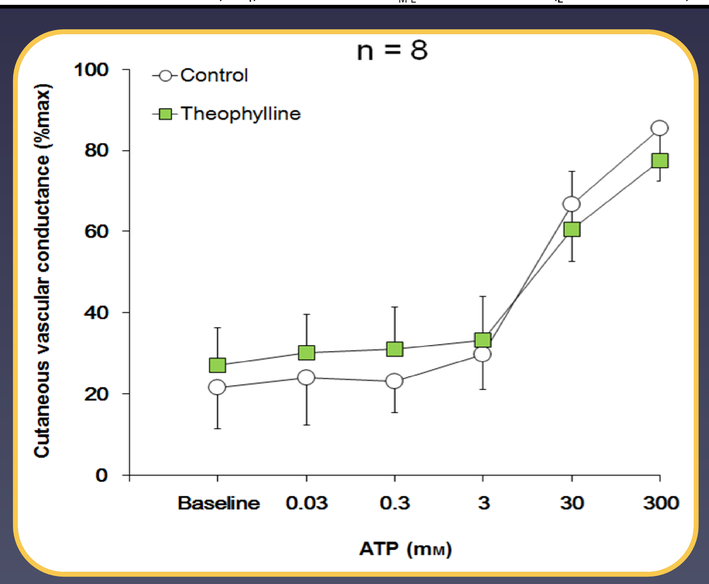


Figure 4: Cutaneous vascular conductance at baseline and during ATP administration from 0.03 to 300 m_M at two skin sites. Sites: 1) lactated Ringer (**Control**) or 2) 4 m_M theophylline (**Theophylline** an adenosine receptor inhibitor).

Discussion

We assessed individual and combined roles of NOS and COX in cutaneous vascular and sweating responses to increased levels of intradermal ATP in humans *in vivo*. It was found that 30 and 300 m_M ATP caused the greatest increase in CVC. Initially, we predicted that cutaneous vasodilation at 30 m_M of ATP would be influenced by inhibition of COX; this was not the case. In accordance with our predictions, CVC was slightly reduced by an inhibition of NOS. Furthermore, increases in CVC were partially mediated by NOS-dependent mechanisms since perfusion of $_L$-NNA and $_L$-NNA + Ketorolac reduced CVC by ~ 20% at 30 m_M ATP compared to Control (Figure 3a). This response was not observed at the ketorolac site. Thus, ATP does not influence the adenosine receptor antagonist and it does not increase sweat rate.

Additionally, ATP can cause activation of P2 receptors and this creates a calcium ion influx. Increased calcium levels induce NOS-dependent cutaneous vasodilation. P2 receptors are found on sensory nerves and it is possible that ATP-mediated cutaneous vasodilation elicited from these sensory nerves.

Adenosine alone can cause pronounced cutaneous vasodilation in humans. Our results demonstrate that CVC did not differ between Control and Theophylline (adenosine receptor inhibitor) at all doses of ATP (Figure 4) indicating that observed cutaneous vasodilation was independent of adenosine-dependent pathways. Although P2 receptors are found in the human sweat gland, activation of these receptors by ATP in humans *in vivo* does not influence human sweating.

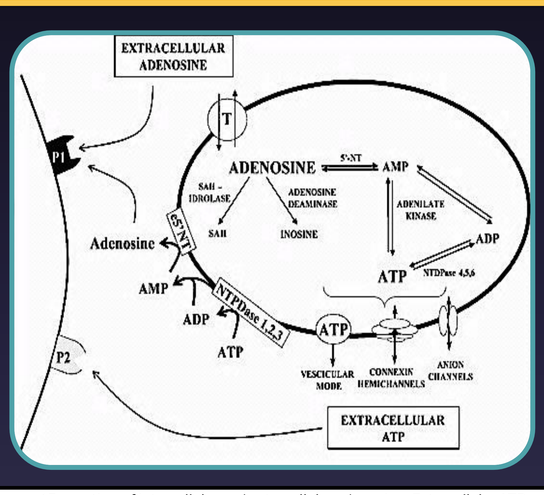


Figure 5. [1]Formation of intracellular and extracellular adenosine. Extracellular ATP binds to P2 purinergic receptors, whereas extracellular adenosine binds to P1 receptors.
[1] Taken from: Pedata, F., Melani, A., Pugliese, A.M., Coppi, E., Cipriani, S., & Traini, C. (2007). The role of ATP and adenosine in the brain under normoxic and ischemic conditions. *Purinergic Signalling, 3,* 299-310.

Applicability and Significance

Patient populations with endothelial damage, reduced bioavailability of ATP and/or hyperhidrosis benefit from assessing the principal pathways involved in ATP-mediated vasodilation and sweating. This is significant for understanding how oxygen delivery and blood flow are impaired within these populations. Future studies are warranted to assess the importance of ATP in modulating cutaneous blood flow and sweating during exercise.

Key References and Acknowledgements

1. Fujii, N., McGinn, R., Paull, G., Stapleton, J.M., Meade, R.D., & Kenny, G.P. (2014). Cyclooxygenase inhibition does not alter methacholine-induced sweating. *Journal of Applied Physiology, 117,* 1055-1062.
2. Mortensen, S.P, Gonzalez-Alonso, J., Bune, L.T., Saltin, B., Pilegaard, H., & Hellsten, Y. (2009). ATP-induced vasodilation and purinergic receptors in the human leg: roles of nitric oxide, prostaglandins, and adenosine. *American Journal of Physiology, 296,* 1140-1148.
3. Wingo, J.E., Brothers, R.M., Del Coso, J., & Crandall, C.G. (2010). Intradermal administration of ATP does not mitigate tyramine-stimulated vasoconstriction in human skin. *American Journal of Comparative Physiology, 298*, 1417-1420.

I would like to thank Dr. Glen P. Kenny, Dr. Naoto Fujii, and Sarah Singh.