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INTRADERMAL ADMINISTRATION OF ATP DOES NOT MITIGATE TYRAMINE-STIMULATED VASOCONSTRICTION IN HUMAN SKIN

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Running Head: ATP and attenuated vasoconstriction

ABSTRACT

Cutaneous vasodilation associated with whole-body heat stress occurs via withdrawal of adrenergic vasoconstriction and engagement of cholinergic "active" vasodilation, the latter of which attenuates cutaneous vasoconstrictor responsiveness. However, the precise neurotransmitter(s) responsible for this sympatholytic-like effect remain unknown. In skeletal muscle, adenosine triphosphate (ATP) inhibits adrenergically-mediated vasoconstriction. ATP also may be responsible for attenuating cutaneous vasoconstriction since it is co-released from cholinergic neurons. The effect of ATP on cutaneous vasoconstrictor responsiveness, however, has not been investigated. Accordingly, this study tested the hypothesis that ATP inhibits adrenergically-mediated cutaneous vasoconstriction. To accomplish this objective, four microdialysis probes were inserted in dorsal forearm skin of 11 healthy individuals (mean ± SD; 35 ± 11 years). Local temperature at each site was clamped at 34 °C throughout the protocol. Skin blood flow was indexed by laser-Doppler flowmetry and was used to calculate cutaneous vascular conductance (CVC; laser-Doppler-derived flux/mean arterial pressure), which was normalized to peak CVC achieved with sodium nitroprusside infusion combined with local skin heating to ~42 °C. Two membranes were perfused with 30 mM ATP while the other two membranes were flow matched via administration of 2.8 mM adenosine to serve as control sites. After achieving stable baselines, 1×10^{-4} M tyramine was administered at all sites while ATP and adenosine continued to be infused at their respective sites. ATP and adenosine infusion increased CVC from baseline by 35 ± 26 $\%CVC_{peak}$ units and by 36 ± 15 $\%CVC_{peak}$ units, respectively (P=0.75). Tyramine decreased CVC similarly (by about one third) at all sites (P<0.001 for main effect and P=0.32 for interaction). These findings indicate that unlike in skeletal muscle, ATP does not attenuate tyramine-stimulated vasoconstriction in human skin.

Keywords: skin blood flow, thermoregulation, cutaneous vasodilation, laser-Doppler flowmetry

INTRODUCTION

Heat stress causes pronounced increases in cutaneous vascular conductance (CVC) that are mediated by the combined effects of withdrawal of sympathetic vasoconstrictor neural activity along with increases in sympathetic cholinergic neural activity, the latter of which is primarily responsible for the large increases in skin blood flow (8, 19). Neurotransmitters are co-released from sympathetic cholinergic nerves with acetylcholine, but acetylcholine is not the primary neurotransmitter causing the dilating effect (12). The precise neurotransmitter(s) responsible for cutaneous active vasodilation remain elusive, however (10, 15, 30, 31, 35).

Besides increased skin blood flow, neurotransmitter(s) responsible for cutaneous active vasodilation also may contribute to attenuated vasoconstriction, leading to severely compromised blood pressure regulation in heat-stressed subjects. Indeed, during a profound hypotensive challenge in individuals subjected to heat stress, even at the point of ensuing syncope, the reduction in CVC is relatively modest such that it remains well above pre-heat stress levels (11, 23, 32). Kellogg et al. (11) attributed this relatively modest decrease in CVC entirely to withdrawal of active vasodilatory tone. Conversely, Shibasaki and colleagues (23) proposed that neurotransmitters released from cutaneous active vasodilator nerves, or "downstream" effects of those transmitters, may cause a sympatholytic effect that inhibits the responsiveness of the cutaneous vasoconstrictor system. Although Shibasaki et al. (23) proposed that nitric oxide may contribute to this sympatholytic effect, other neurotransmitters co-released from cholinergic neurons may contribute to the attenuation of cutaneous vasoconstrictor responsiveness in heat-stressed subjects.

Adenosine triphosphate (ATP) may contribute to the attenuation of cutaneous vasoconstrictor responsiveness in heat-stressed subjects since it is co-released from multiple

nerve types in humans and animals (1, 2, 5, 18, 29, 36), has sympatholytic effects in human skeletal muscle (13, 21), and dilates the cutaneous vasculature (6). However, in order for ATP to attenuate the effectiveness of the cutaneous vasoconstrictor system in heat-stressed subjects, it must be co-released from sympathetic cholinergic nerves during a heat stress. Unfortunately, perhaps due to the lack of an available selective ATP antagonist safe for use in humans, it remains unknown whether ATP contributes to cutaneous vasodilation during thermal exposure. Nevertheless, if ATP has sympatholytic effects in skin similar to effects in human skeletal muscle, this could provide the basis for future work investigating a possible role of ATP in contributing to cutaneous vasodilation during whole-body heat stress. Accordingly, the purpose of this study was to test the hypothesis that ATP inhibits adrenergically-mediated cutaneous vasoconstriction.

METHODS

Subjects

Eleven healthy individuals (8 men and 3 women) volunteered to participate. Their mean ± SD age, height, and weight were 35 ± 11 y, 174 ± 6 cm, and 72 ± 11 kg, respectively. The phase of the menstrual cycle was not normalized across female subjects. Study and informed consent approval was given by the institutional review boards at the University of Texas Southwestern Medical Center at Dallas and at Texas Health Presbyterian Hospital Dallas, and subjects provided written informed consent prior to enrolling.

Instrumentation

Upon arrival at the laboratory, subjects rested supine while 4 microdialysis membranes (Bioanalytical Systems, West Lafayette, IN) were inserted approximately 4-5 cm apart in dorsal

forearm skin. Each membrane was initially perfused with lactated Ringer's solution (Baxter, Deerfield, IL) at a rate of 2 μL/min via a perfusion pump (Harvard Apparatus, Holliston, MA) while insertion trauma associated with membrane placement subsided (minimum 90 min). During this time, each site was instrumented with a local heater (PF 450, Perimed, North Royalton, OH) covering approximately 7 cm^2 and housing a laser-Doppler flow probe (Model DP7a, Moor Instruments, Wilmington, DE) used to provide an index of skin blood flow. A thermocouple (Type T, Omega Engineering, Stamford, CT) was placed between the skin and local heater to monitor local skin temperature, and a cuff was placed around the arm contralateral to the arm where the microdialysis membranes were inserted to intermittently measure blood pressure from the brachial artery using electrosphygmomanometry (Tango, SunTech Medical Instruments, Raleigh, NC).

Procedures

After a period of at least 90 min to allow the hyperemic response associated with membrane placement to subside, all sites were locally heated to 34 °C for approximately 20 min. This was done to standardize skin temperature across sites since skin temperature is known to affect skin blood flow (3). While sites were maintained at 34 °C, 30 mM ATP (A2383, Sigma-Aldrich, Inc., St. Louis, MO) was administered at 2 sites while 2.8 mM adenosine (42734A, Abraxis Pharmaceuticals Products, Schaumburg, IL) was administered at the remaining 2 sites, which served as flow controls. The dose of ATP utilized was selected based on pilot testing which revealed that 30 mM ATP caused cutaneous vasodilation to a comparable level as that elicited by 2.8 mM adenosine. Adenosine was chosen as the control condition because it has been shown to have no effect on cutaneous vasoconstriction (24, 25). Once skin blood flow was elevated and stable, 2 doses of tyramine (a monoamine compound that causes the release of

stored monoamines known to cause vasoconstriction, such as norepinephrine, from sympathetic nerve terminals) were sequentially administered at all sites while each site continued to receive its respective dose of ATP or adenosine. Each dose of tyramine (1×10^{-4} M followed by 1×10^{-2} M) was administered for ~12 min at 2 μL/min. In some subjects, a paradoxical cutaneous vasodilation (34) occurred in conjunction with 1×10^{-2} M tyramine, without respect to whether the site was co-infused with adenosine or ATP, so only data during 1×10^{-4} M tyramine administration were analyzed. After tyramine administration, all sites were locally heated to ~ 42 °C, coupled with administration of 28 mM sodium nitroprusside (SNP; a nitric oxide donor), to achieve peak cutaneous vasodilation. Use of the term, "peak" cutaneous vasodilation was chosen since administration of SNP after tyramine-mediated vasoconstriction likely resulted in submaximal cutaneous vasodilation. Since both ATP- and adenosine-treated sites received the same doses of tyramine, any residual effects of tyramine during local heating and SNP administration should have been uniform across sites. This was confirmed by similar absolute peak CVCs at each site (see Results below).

Data Analysis

Data were acquired continuously at a sampling rate of 50 Hz using a data acquisition system (Biopac, Santa Barbara, CA). Data from the final minute of each respective condition (i.e., before ATP and adenosine administration, before tyramine administration after adenosine and ATP administration, and at the end of 1×10^{-4} M tyramine administration) were averaged and analyzed. Mean arterial blood pressure (MAP) was calculated as 1/3 pulse pressure + diastolic pressure and used in the calculation of CVC (laser-Doppler-derived flux/MAP). CVC data were then normalized to peak (%CVC_{peak}) achieved during local heating combined with SNP administration. Of the 4 microdialysis sites within a given subject, data from the ATP site and

the adenosine site that most closely matched each other in terms of $\%CVC_{peak}$ before tyramine administration were statistically analyzed. A 2-way (site × condition) repeated measures analysis of variance was used to compare CVC as well as local skin temperature responses between adenosine and ATP sites before and after tyramine administration. Paired samples t-tests were used to compare CVC between sites before drug infusion and during SNP administration, as well as to compare the increase in CVC to drug infusion between sites. Statistical analyses were performed using SPSS v. 15.0 for Windows (SPSS, Inc., Chicago, IL). A P-value < 0.05 was considered statistically significant. Data are expressed as means ± SD.

RESULTS

Figure 1 shows a representative tracing of the skin blood flow response at baseline, during local heating to 34 °C, during ATP and adenosine administration, and during 1×10^{-4} M tyramine administration. Pre-drug (after local heating to 34 °C but prior to ATP or adenosine administration) CVC was similar between sites (21 ± 16 $\%CVC_{peak}$ and 20 ± 12 $\%CVC_{peak}$ for the sites to receive ATP and adenosine, respectively; P = 0.51). Likewise, the increase in CVC from baseline during drug infusion was not different between sites (ATP: +35 ± 26 $\%CVC_{peak}$ units; Adenosine: +36 ± 15 $\%CVC_{peak}$ units; P = 0.75). Peak CVC achieved with SNP administration and local heating to 42 °C also was not different between sites (ATP: 2.7 ± 0.8 perfusion units/mm Hg; Adenosine: 2.7 ± 0.7 perfusion units/mm Hg; P = 0.80). Local skin temperature was consistent between sites throughout tyramine administration (pre-tyramine, adenosine-treated site: 33.8 ± 0.3 °C, ATP-treated site: 33.9 ± 0.3 °C; tyramine, adenosine-treated site: 33.9 ± 0.3 °C, ATP-treated site: 33.9 ± 0.4 °C; P = 0.55 for interaction and P = 0.35 for site main effect). Tyramine was effective in reducing CVC at both the ATP and adenosine

treated sites ($P < 0.001$; see Figure 2). However, the magnitude of the reduction in CVC was not different between sites ($P = 0.32$ for interactive comparison).

DISCUSSION

This study tested the hypothesis that ATP attenuates tyramine-mediated cutaneous vasoconstriction, given prior studies showing that ATP attenuates tyramine-mediated vasoconstriction in skeletal muscle (21, 22). Contrary to the findings in skeletal muscle, the primary finding was that ATP did not attenuate cutaneous vasoconstriction to exogenous tyramine administration when compared to the vasoconstrictor response at sites that were flow matched via adenosine administration.

The precise mechanism for the sympatholytic action of ATP in skeletal muscle remains unclear, but investigators hypothesize that endothelium-derived hyperpolarizing factors released as a result of ATP binding to P_2 purinergic receptors on the endothelium trigger a signaling cascade that activates K_{ATP} channels (21). These channels have been implicated in the attenuation of α-adrenergic vasoconstriction during exercise (9, 27). Some might argue that the metabolic byproducts of ATP degradation, like adenosine diphosphate, adenosine monophosphate, and adenosine, are primarily responsible for the attenuation of adrenergic vasoconstriction in skeletal muscle that has been attributed to ATP. However, Rosenmeier et al. showed that ATP, not its dephosphorylated metabolites, is the primary substance responsible for the sympatholytic effect of ATP (22). Furthermore, whole limb studies have shown adenosine infusion does not affect tyramine-mediated vasoconstriction (4, 22, 28), whereas ATP infusion blunts adrenergic vasoconstriction (7, 22). Finally, adenosine did not affect α_1 or α_2 adrenoceptor-mediated vasoconstriction in the forearm, whereas ATP completely abolished α-

adrenoceptor-mediated vasoconstriction (13). Taken together, these findings, coupled with robust cutaneous vasoconstriction at adenosine-treated sites in a prior research (25), argue against the notion that the absence of a difference in response to tyramine between ATP and adenosine sites (Figure 2) was because of ATP and adenosine having comparable sympatholytic properties in human skin.

Kirby et al. (13) reported the sympatholytic effect of ATP in the exercising forearm to be graded such that the greatest magnitude of sympatholysis occurred with the highest doses of ATP, while a sympatholytic effect did not occur at the lowest dose of ATP. Given those findings, one could argue that the dose of ATP in the current study was perhaps too low to attenuate cutaneous vasoconstriction to tyramine. However, in the current study the concentration of ATP (3×10^{-2} M; equivalent to 3×10^{7} nM) administered directly to the dermal interstitial spaces was greater than the highest dose used in the aforementioned study by Kirby et al. (13), even when taking into account that the relative delivery of the drug was likely between 10% and 30% of the perfused concentration. The fact that tyramine-mediated cutaneous vasoconstriction was unaffected by ATP, despite a concentration of ATP higher than that in the study by Kirby et al. (13) and sufficient to cause considerable vasodilation (Figure 2), supports the notion that ATP administered to the dermal interstitium does not mitigate tyramine-stimulated vasoconstriction in human skin.

A limitation of the current study is the inability to determine the extent to which ATP acted on P2Y and P2X receptors in the skin. *In vitro* studies in animals have demonstrated the vasodilatory effect of ATP, mediated by P2Y purinergic receptors, is reduced when high doses are administered because ATP binds to P2X receptors and causes the release of endothelial-derived contracting factors (16, 20). However, *in vivo* intravascular data in humans do not

support this assertion (6, 20). Rongen et al. found that intra-arterial infusions of a high dose of ATP [up to 1000 μg/(100 mL forearm·min^{-1}) for 5 min] resulted in vasodilation that was not reduced by the release of endothelial-derived contracting factors (20). While the timing of the vasoconstrictor responses to tyramine does not support the action of ATP on P2X receptors contributing to this vasoconstriction (see Figure 1), we cannot exclude the possible effect of the concentration of ATP used in the current study stimulating P2X receptors and perhaps masking a sympatholytic effect of ATP. Additionally, this relatively high concentration of ATP [when compared to an estimated interstitial concentration of 0.3 μM (17)] may downregulate membrane-bound P2Y receptors, perhaps attenuating a sympatholytic effect of ATP.

Although the primary objective of this investigation was to identify whether ATP has sympatholytic effects in skin similar to that previously reported in muscle (13, 21), the presumption is that ATP is released from cutaneous sympathetic cholinergic nerves. This presumption remains unconfirmed, perhaps because of the unavailability of an ATP antagonist approved for use in humans that would be employed in such an evaluation. Nevertheless, in the absence of complete knowledge regarding ATP as a cutaneous neurotransmitter, the obtained findings remain valuable in excluding ATP as a substance responsible for previously observed sympatholytic effects associated with cutaneous active vasodilation (14, 23, 26, 32).

Finally, one may surmise that perhaps the magnitude of vasoconstriction to tyramine may be so profound that a sympatholytic effect of ATP is masked. However, this is unlikely given that the decrease in skin blood flow to exogenous tyramine administration was relatively small (see Figure 2) when compared to the potential for the skin to constrict to exogenous adrenergic agents such as norepinephrine (32-34). Furthermore, larger doses of tyramine, relative to that

used in the present study, leads to greater vasoconstriction (34). Thus, it is unlikely that a sympatholytic effect of ATP was masked by a profound vasoconstrictor stimulus.

Perspectives and Significance

These data demonstrate that, unlike in skeletal muscle, ATP administered to the dermal interstitium does not have sympatholytic effects in human skin. In light of previous findings showing substances associated with cutaneous active vasodilation attenuate cutaneous vasoconstrictor responsiveness (14, 23, 26, 32), it is unlikely that ATP from intradermal sources contributes to this sympatholytic-like effect. Future studies are warranted to identify whether ATP from other sources (e.g., skeletal muscle) is capable of attenuating adrenergically-mediated cutaneous vasoconstriction.

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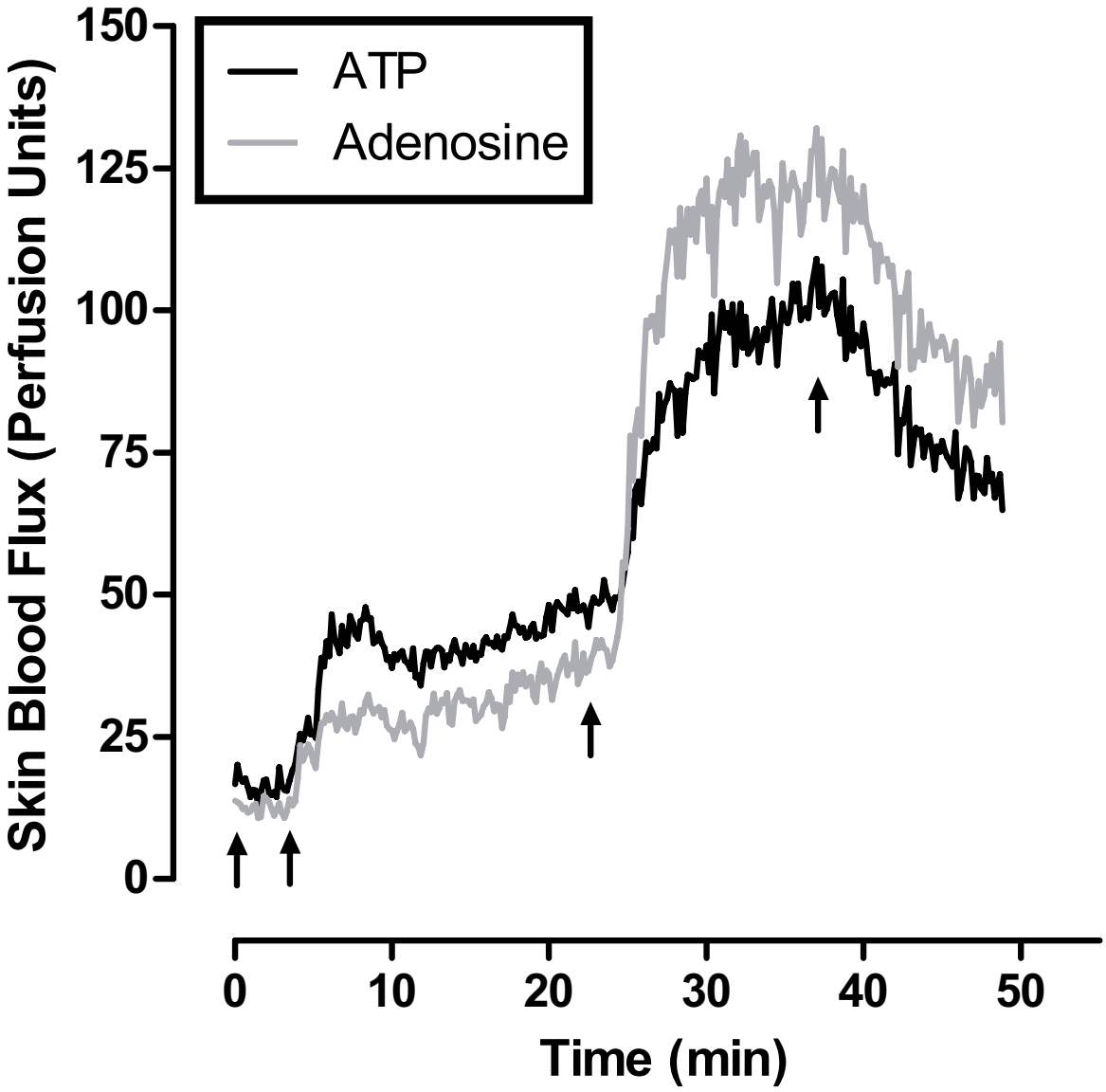
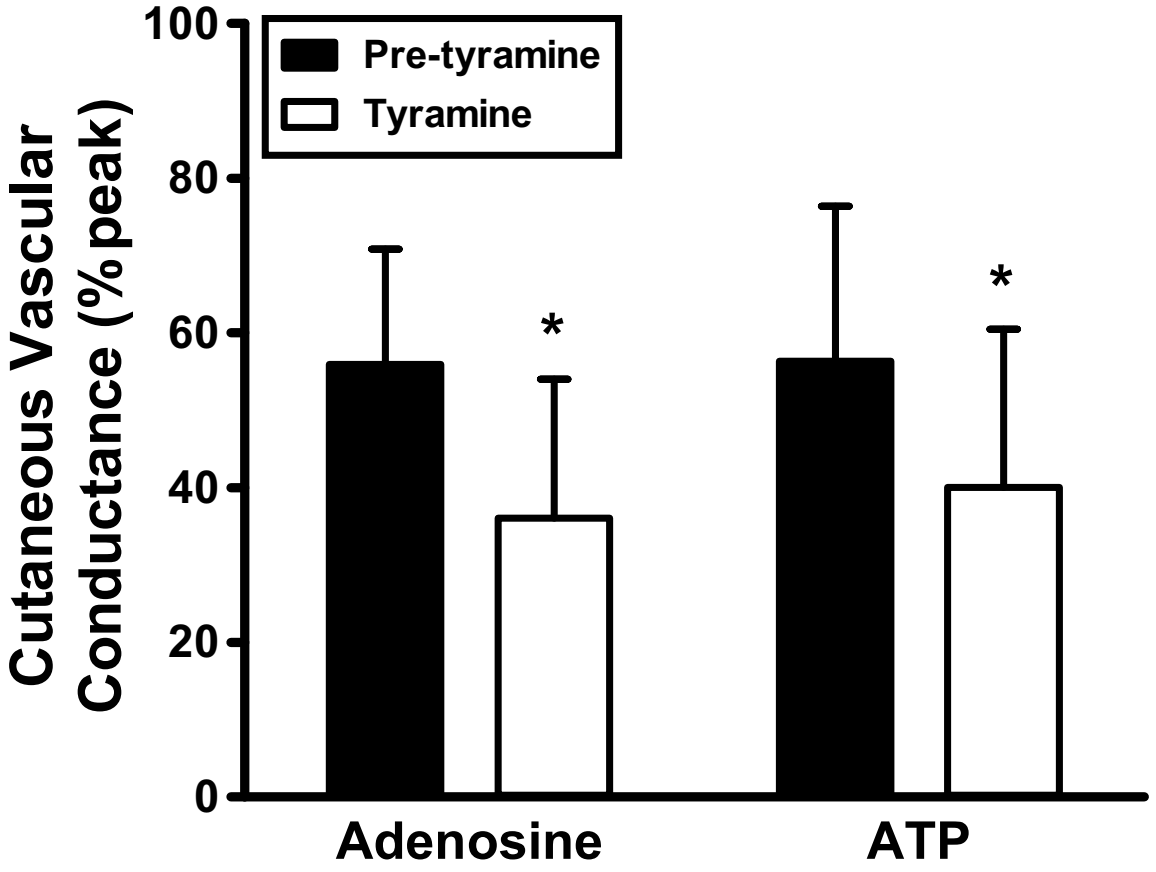
FIGURE LEGENDS

Figure 1. Representative tracing illustrating skin blood flow responses during baseline, local heating to 34 °C, ATP and adenosine infusion, and 1×10^{-4} M tyramine infusion (arrows are in chronological order).

Figure 2. The effects of tyramine on cutaneous vascular responses (normalized to peak cutaneous vascular conductance; CVC) at sites previously dilated with either ATP or adenosine. Exogenous tyramine decreased CVC at both ATP and adenosine sites (main effect denoted by *; $P < 0.001$), but the magnitude of cutaneous vasoconstriction was unaffected by ATP relative to the flow-matched adenosine site (interaction effect; $P = 0.32$).


ATP
Adenosine
Skin Blood Flux (Perfusion Units)
150
125
100
75
50
25
0
0
10
20
30
40
50
Time (min)


Cutaneous Vascular Conductance (% peak)
100
80
60
40
20
0
Pre-tyramine
Tyramine
*
*
Adenosine
ATP