Acidosis attenuates P2X purinergic vasoconstriction in skeletal muscle arteries

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Kluess, Heidi A., John B. Buckwalter, Jason J. Hamann, and Philip S. Clifford. Acidosis attenuates P2X purinergic vasoconstriction in skeletal muscle arteries. Am J Physiol Heart Circ Physiol 288: H129–H132, 2005. First published September 16, 2004; doi:10.1152/ajpheart.00574.2004.—Vasoconstriction via α2-receptors is known to be sensitive to acidic pH, but little is known about the pH sensitivity of P2X receptors. ATP is a cotransmitter released with norepinephrine from the sympathetic nervous system and causes vasoconstriction via P2X purinergic receptors on vascular smooth muscle. We hypothesized that reductions in pH would attenuate P2X-mediated vasoconstriction in iliofemoral artery rings. Twenty-five rats were killed, and the iliac and femoral arteries were dissected out and placed in modified Krebs-Henseleit buffer. The arteries were cut into 2-mm sections and mounted in an organ tissue bath. Tension (g) was measured during a potassium chloride and norepinephrine challenge (maximal tension). Dose-response curves were fit with nonlinear regression analysis to calculate the EC50 and slope. The peak tension with α,β-methylene ATP was lower during pH 7.0 (1.37 ± 0.09 g) compared with pH 7.8 (1.90 ± 0.12 g). EC50 was highest with pH 7.4 (−5.38 ± 0.18 log M α,β-methylene ATP) and lowest with pH 7.0 (−4.9 ± 0.10 log M α,β-methylene ATP). The slopes of the dose-response curves were not different. Pyridoxal phosphate-6-azo(benzene-2,4-disulfonic acid) abolished contraction caused by the addition of α,β-methylene ATP (n = 6). There was no effect of pH on phenylephrine dose-response curves. These data indicate that the vasoconstrictor response to α,β-methylene ATP is sensitive to pH and that lower pH attenuates the response of P2X purinergic receptors.

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maximal tension produced during the potassium chloride and norepinephrine challenge. Dose-response curves were fit with nonlinear regression analysis to calculate the EC50 of peak tension and the slope. Percent maximal values were used for regression analysis. Statistical analysis of minimum tension, peak tension, EC50, and the slope for α,β-methylene ATP and phenylephrine was performed with an one-way repeated-measures analysis of variance followed by Tukey’s post hoc test when appropriate.

RESULTS

Baseline tensions were not different at pH 7.8, 7.4, and 7.0 (0.47 ± 0.02 g; P > 0.05). Peak tension produced by α,β-methylene ATP at pH 7.8 (1.90 ± 0.12 g) and 7.4 (1.83 ± 0.11 g) were significantly greater than peak tension produced at pH 7.0 (1.37 ± 0.09 g; P < 0.05). Figure 1 summarizes the mean tension (%maximal) response to all doses of α,β-methylene ATP at pH 7.8, 7.4, and 7.0. EC50 for the α,β-methylene ATP curves was significantly lower with pH 7.0 compared with pH 7.4 but not significantly different from pH 7.8 (Fig. 2). The slope of the dose-response curve was not affected by changes in pH (1.36 ± 0.07 %maximal tension/log M α,β-methylene ATP; P > 0.05). Addition of the P2 antagonist PPADS to the bath abolished the contractile response to 10⁻⁵, 10⁻⁴, and 10⁻³ M α,β-methylene ATP (Fig. 3).

There was no effect of pH on peak tension (1.92 ± 0.12 g; P > 0.05) produced during phenylephrine dose-response curves (see Fig. 4). The EC50 (−6.33 ± 0.06 log M phenylephrine; P > 0.05) and slope (0.87 ± 0.03 %maximal tension/log M phenylephrine; P > 0.05) were not different at pH 7.8, 7.4, or 7.0.

DISCUSSION

The major finding of this study is that vasoconstriction to α,β-methylene ATP was sensitive to hydrogen ions in the rat iliofemoral artery. In contrast, we found no effect of pH on reactivity to the α₁-agonist phenylephrine, suggesting that pH does not have a nonspecific effect on the vascular smooth muscle. To our knowledge, there have been no previous studies investigating the effect of pH on purinergic vasoconstriction in skeletal muscle arteries. These data demonstrate that the vasoconstrictor response to α,β-methylene ATP is sensitive to extracellular hydrogen ions and that lower pH attenuates the response to P2X purinergic receptors.

Consistent with previous work in cloned receptors, we found a rightward shift in the α,β-methylene ATP dose-response curve, with pH 7.0 resulting in a decrease in EC50 (10, 22). This finding is in contrast to others who have shown an increased sensitivity of P2X receptor response to acidification (6, 10, 14, 15). The apparently conflicting results of these studies may be due to different P2X receptor subtypes in different tissues (22). Other studies have shown that most P2X subunits are sensitive to pH, with P2X1, P2X3, and P2X4 activity being attenuated by low pH, whereas P2X2 and P2X2/3 activity are enhanced by low pH (22). Therefore, our findings...
P2X ion pore (22). Stoop et al. (22) suggested that a decrease in ATP-activated current with decreasing pH in cloned P2X2 receptors may be attenuated by protonation. Unique to this study, we found a significant attenuation of peak tension at pH 7.0. These data are in contrast to those of Stoop et al. (22) and Li et al. (10), who found no change in peak ATP-induced activity at low pH. It is possible that the differing results are due to differences in P2X receptor subtypes studied (10, 22). Nevertheless, our data from rodent skeletal muscle arteries suggest that hydrogen ions may reduce ion pore permeability. This possibility needs to be confirmed by further patch-clamp experiments.

One might consider that mechanisms not specific to the P2X receptor may be involved in reduced tension development with acidosis. They include activation of ATP-sensitive potassium channels and hydrogen ion interference with the contractile machinery (1, 18, 23, 24). However, the P2 antagonist PPADS abolished tension produced by α,β-methylene ATP, indicating that vasoconstriction to α,β-methylene ATP was mediated solely by purinergic receptors. In addition, we found no effect of pH on tension produced by the α1-agonist phenylephrine. These data argue against the possibility of a nonspecific effect of hydrogen ions on modifying channels or proteins not related to the P2X receptor (1).

The physiological significance of these findings is that they provide a potential mechanism to explain observations regarding skeletal muscle vasculature during exercise (4). Recently, our lab demonstrated (5) that P2X receptors mediate tonic vasoconstriction in exercising skeletal muscle. However, the presence of vasoconstriction to exogenously administered α,β-methylene ATP was attenuated during heavy exercise. We hypothesized that the mechanism of this attenuation may be related to a change in the chemical environment during exercise. Heavy exercise can result in decreased pH, and, therefore, the current study provides a plausible mechanism for these observations (4, 5, 13, 21).

In conclusion, we found a rightward shift in the dose-response curve and a decrease in peak tension with α,β-methylene ATP and acidification. The α-agonist dose-response curve was unaffected by pH. To our knowledge, this is the first study to investigate the sensitivity of P2X-mediated vasoconstriction with extracellular acidification in skeletal muscle arteries. Although the mechanism requires further investigation, the results show that lower pH attenuates the vasoconstrictor response to P2X purinergic receptors. These results imply that during conditions such as exercise and peripheral arterial insufficiency where pH is reduced, diminished P2X-mediated responsiveness may influence vascular tone.

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