Control of blood pressure during exercise by gut-derived toxins

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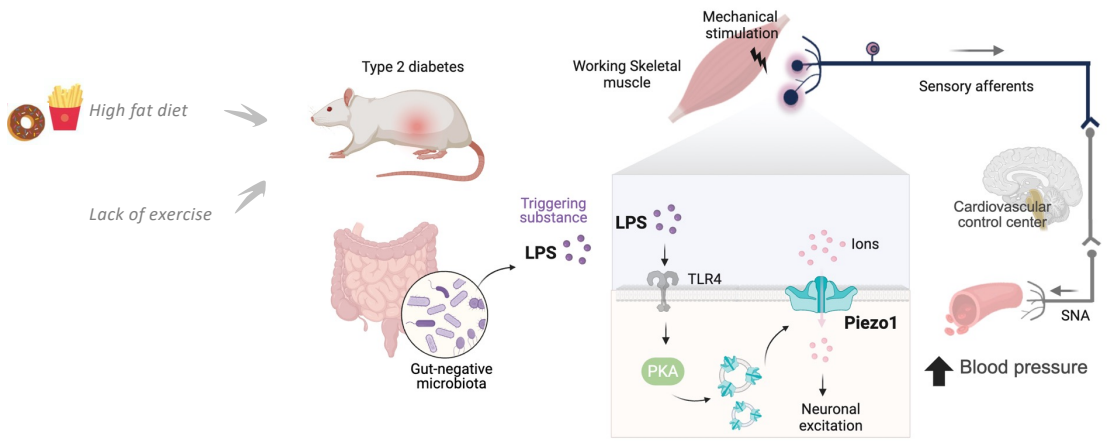
Graphic abstract



LPS: lipopolysaccharide, PKA: protein kinase A, SNA: sympathetic nerve activity, TLR4: toll-like receptor 4.

Background and Purpose

An abnormal mechanoreflex function from skeletal muscle significantly contributes to the heightened circulatory response during exercise in type 2 diabetes mellitus (T2DM). However, the underlying mechanisms remain unclear. Piezo1 channels are known to play crucial roles in touch and pain sensation. More importantly, a recent study suggested that Piezo channels may significantly contribute to the generation of mechanoreflex activity in healthy rats. Lipopolysaccharide (LPS), a *toxic* substance produced by gut-negative bacteria, is increased in patients with T2DM. a receptor of LPS, toll-like receptor 4 (TLR4), drives the innate immune response via Piezo1 activation in macrophages. Thus, it is logical to further suggest that LPS may sensitize sensory afferents in skeletal muscle via activation of mechanoreceptor ion channels leading to increased mechanoreflex responsiveness. Accordingly, this study hypothesized that the abnormally enhanced EPR response to exercise in T2DM is mediated by mechanoreflex-induced exaggerations that result, at least in part, from augmentations in the responsiveness of mechanosensitive-muscle afferents via sensitized Piezo1 channel activity. To test this hypothesis, Piezo1 protein expression in the plasma membrane with LPS exposure in HEK293 cells *in vitro*.

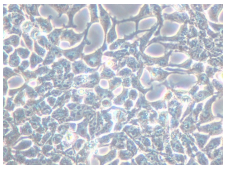
Methods

Cell Culture Treatment

The human embryonic kidney cell line, HEK293, was maintained in a humidified cell culture incubator at 37 °C with a 95% air, 5% CO_2 atmosphere. Cells were incubated in 1000 ng/mL LPS (Sigma-Aldrich) or PBS as a vehicle supplemented with DMEM containing 1% PS for 30 min at 37 °C. For mechanistic experiments, HEK293 cells were pretreated with the protein kinase C (PKC) inhibitor Bisindolymeleimide I (B5781, Tokyo Chemical Industry) or the protein kinase A (PKA) inhibitor PKI(5-24) (15996, Cayman) for 60 min, and then were exposed to 1000 ng/mL LPS for 30 min. After incubation, cells were harvested. Plasma membrane and cytosol were fractionated by using a Plasma Membrane Protein Extraction Kit (ab65400, abcam).

Western blotting

HEK293 cells were lysed in a RIPA buffer (89901, Thermo Scientific) or homogenization buffer for plasma membrane fraction (ab65400, abcam). The samples were incubated with anti-Piezo1 (1:1000 dilution; ab128245, abcam), GAPDH (1:1000 dilution; #2118, Cell Signaling) and Na^+/K^+ ATPase (1:1000 dilution; ab7671, abcam) at 4°C overnight.



HEK293 cell

Results

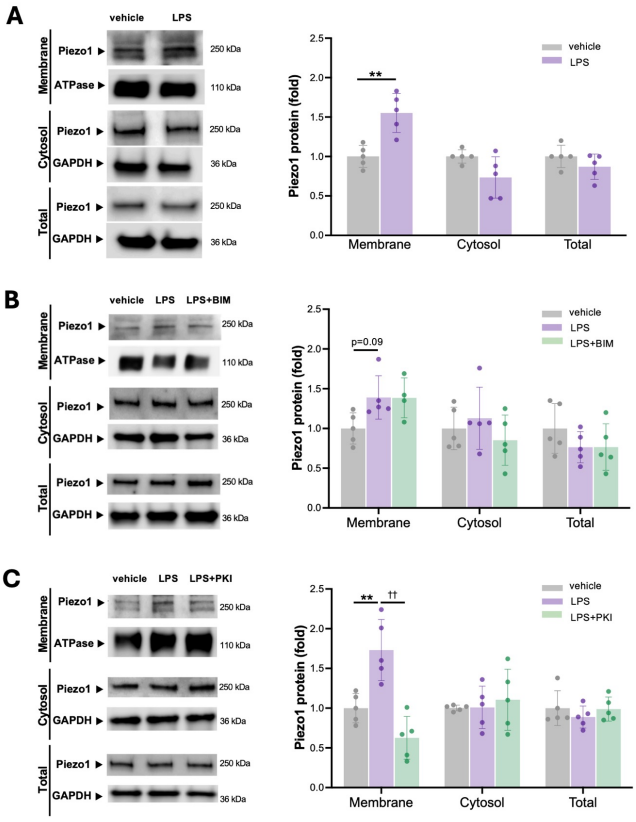


Figure 1. Lipopolysaccharide (LPS)-induced translocation of Piezo1 channel via PKA, but not PKC, to plasma membrane in HEK293 cells. A, Representative western blots and quantified expression of Piezo1 protein in the plasma membrane, cytosol and cytoplasm to a vehicle and 1000 ng/mL LPS treatments in HEK293 cells. B, Representative western blots and quantified expression of Piezo1 protein in plasma membrane, cytosol and total fraction to a vehicle, 1000 ng/mL LPS treatments with/without Bisindolylmaleimide I (BIM), protein kinase C (PKC) inhibitor, in HEK293 cells. C, Representative western blots and quantified expression of Piezo1 protein in plasma membrane, cytosol and cytoplasm to a vehicle, 1000 ng/mL LPS treatments with/without PKI (5-24), protein kinase A (PKA) inhibitor, in HEK293 cells. Values are means ± SD, ** $P < 0.01$ compared to vehicle. †† $P < 0.01$ compared to LPS.

Conclusion

These findings suggest that LPS potentially induces excitatory neuronal activity via the Piezo1 channel in mechanosensitive muscle afferents, mediated by LPS-evoked Piezo1 membrane translocation via the PKA signaling pathway.

Acknowledgements & Correspondence

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