THE INHIBITION OF PTP1B REVERSES IMPAIRED VAGAL AFFERENT SENSITIVITY CAUSED BY DIET-INDUCED OBESITY, REDUCING NITRIC OXIDE AND 2 PORE DOMAIN K⁺ CONDUCTANCE

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Background: We have demonstrated that diet-induced obesity impairs vagal afferents and that the effects of high concentrations of leptin on vagal afferents in vitro, mimic the effects of obesity. One of downstream molecules in leptin signaling is protein tyrosine phosphatase 1B (PTP1B), which plays a role in negative regulation.

Aims: We hypothesized that inhibition of PTP1B would prevent an impairment of vagal afferents caused by diet-induced obesity/hyperleptinemia

Methods: Diet-induced obese (DIO) mice were fed diets composed of 60% or 10% kCal fat for 12-16 weeks. Nodose neurons from DIO mice or standard diet fed mice were incubated overnight with leptin (100nM) and PTP1B inhibitor (10mM). Current and voltage clamp were performed to assess membrane excitability and two-pore domain K⁺ (K2P) conductance, respectively. NO was measured in culture media using Nitrate/Nitrite fluorometric Assay kit. Media was collected after cell incubation for 24 hrs.

Results: Leptin (100nM) incubation reduced the excitability of vagal afferents. PTP1B inhibitor reversed this inhibitory effect of leptin. PTP1B inhibitor significantly decreased rheobase (100.8 ± 18.6 pA, n=12, leptin) vs. 64.7 ± 5.2 pA (n=15, Leptin + PTP1B inhibitor), *p=0.0497) and significantly increased input resistance (353.8 ± 36.2 MΩ (n=12, leptin) vs. 508.2 ± 52.7 MΩ (n=15, Leptin + PTP1B inhibitor), *p=0.0305). Leptin significantly increased K2P conductance (0.280 ± 0.022 nS (n=17, control) vs. 0.377 ± 0.035 nS (n=16, leptin), *p=0.0108). Inhibition of PTP1B significantly decreased the conductance (0.249 ± 0.013 pA (n=14, Leptin + PTP1B inhibitor), **p=0.0028) in leptin-incubated neuron. In mice fed a HFF diet, there is reduction in vagal excitability, which was reversed by the inhibition of PTP1B. PTP1B inhibitor significantly decreased rheobase (112.1 ± 14.6 pA (n=14, leptin) vs. 70.8 ± 8.1 pA (n=12, HFF + PTP1B inhibitor), *p=0.0268). Rheobase was not significantly different in LFF. Inhibition of PTP1B significantly decreased K2P conductance both in LFF and HFF neurons (LFF; **p=0.0050; HFF; **p=0.0041). PTP1B inhibitor significantly reduced NO fluorescence in leptin-incubated media (33.1 ± 2.2 (Leptin) vs. 12.2 ± 1.0 (Leptin+PTP1B inhibitor), ***<0.0001, n=6) and in neurons from HFF mice (43.4 ± 0.7 (HFF) vs. 29.9 ± 0.6 (HFF+PTP1B inhibitor), ***<0.0001 n=6), but not in LFF (27.8 ± 1.3 (LFF) vs. 25.8 ± 0.8 (LFF+PTP1B inhibitor), NS n=6).

Conclusions: Inhibition of PTP1B reverses the effects of HFF and elevated leptin on NO production and K2P conductance in nodose ganglion neurons. Consistent with this, the inhibitory effects of leptin and diet-induced obesity on the excitability of vagal afferent neuron were also reversed by PTP1B blockade. We suggest PTP1B may be a pivotal regulator and potential drug target in obesity.
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