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Central regulation of airway reactivity by insulin as a possible mechanism underlying obesity-associated asthma

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Obesity is a major risk factor for asthma. Several researches suggests that obese related asthma phenotype do not necessarily involves inflammation (2). Recent study showed that inhibition of leptin action at the brainstem level reduces airway diameter in obese mice (3). In the present study we aimed to investigate whether insulin regulates airway reactivity through the airway related pre-ganglionic neurons and whether it contributes to innate airway hyperreactivity in hyperinsulinaemic obese mice.

Adult male C57BL/6J mice received insulin (0.02, 0.2 and 2 µU) via intracerebroventricular (ICV) in the presence or in the absence of PI3-kinase inhibitor wortmannin (30nM) or the ERK inhibitor PD98059 (100µM) 30min before the beginning of the measurements of pulmonary mechanics, that were done using the forced oscillation technique with a Flexivent (Scireq, CA) according to previously described(2). Dose-response curves to inhaled methacholine (6, 12.5, 25 and 50 $mg.ml^{-1}$) were performed and the following mechanical parameters were assessed: tissue and respiratory system resistances (Rn, Gtis and R, respectively) and tissue and respiratory system elastances (Htis and Ers, respectively). Insulin effects were also evaluated in homozygous vesicular acetylcholine transporter (VAChT) knockdown mice ($VAChT^{-/-}$) (4). Wild-type and toll-like receptor 4 knockout mice ($TLR4^{-/-}$) were fed with a high-fat diet for 12 weeks to induce obesity and, after that, pulmonary mechanics, lung histology (hematoxylin and eosin) and the endurance test were performed accordingly to previously described(3). *In vitro* concentration-response curves to methacholine in isolated bronchus were also done(4). One-way analysis of variances (ANOVA) followed by a Tukey test was used in all groups. $p < 0.05$ was accepted as significant.

ICV insulin infusion increased airway responsiveness as evidenced by the increase in all above mentioned mechanical parameters at 0.2 and 2 µU insulin concentrations ($p<0.05$). PD98059 inhibited insulin-induced increases in airway responsiveness ($p<0.05$), whilst wortmannin did not modify those parameters. Insulin failed to increase airway responsiveness in $VAChT^{-/-}$ mice. Obese mice were insulin resistant and hyperinsulinaemic and showed enhanced responsiveness to methacholine ($R=2.94 \pm 0.7 cmH_2O$, $p<0.05$) in comparison to lean mice ($R=0.93 \pm 0.1 cmH_2O$). Obese mice also showed reduced endurance (305 ± 30.4m, $p<0.001$) compared to lean mice (1551 ± 103.6m) as measured by their ability to run on a treadmill. No inflammatory infiltration was detected in the lungs from obeses. In vitro contractile-responses to methacholine did not change in obese mice. Although maintained similar body weight (38.33 ± 3.18g) obese $TLR4^{-/-}$ mice displayed improved endurance (690 ± 51m $p<0.05$), enhanced insulin sensitivity and reduced insulin levels and airways

responsiveness ($p<0.05$) in comparison with wild type obese mice.

In conclusion, our data suggests that insulin increases airway reactivity by stimulating airway-related cholinergic pre-ganglionic fibers through the activation of ERK pathway, thereby increasing parasympathetic outflow into the lungs. Our data also shows that this pathway is likely to contribute to the innate airway hyperreactivity showed by obese mice.

(1) Wenzel SE, Nat Med 18:716, 2012; (2) Arteaga-Sollis E *et al*, Cell Metab 17:35, 2013; (3) Prado VF *et al*, Neuron 51:601, 2006; Leiria LO et al, Br J Pharmacol 163:1276, 2011.