Involvement of endogenous adenosine via A_{2B} receptors in colonic dysmotility associated with high fat diet-induced obesity

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Obesity, defined as the accumulation of excess body fat, is a major component of metabolic syndrome, often related to chronic disorders such as type 2 diabetes, cardiovascular diseases and cancer (Mushref and Srinivasan, 2013). Of note, obesity is also associated with gastrointestinal dysfunctions, including altered gastric emptying, intestinal dysmotility and constipation (Xing and Chen, 2004). In this context, there is an increasing evidence supporting the involvement of adenosine A_{2B} receptors ($A_{2B}R$) in altered tissue functions associated with diabetes and obesity (Chiu and Freund, 2014; Antonioli et al., 2015). However, the contribution of adenosine to enteric dysmotility associated with obesity has not been investigated. The aim of the present study was to examine the role played by $A_{2B}R$ in the alterations of colonic neuromuscular functions in a mouse model of diet-induced obesity (DIO).

C57BL/6 male mice (age 6 weeks) were fed with regular diet (RD; 9% calories from fat) or high fat diet (HFD; 60% calories from fat) for 8 weeks to obtain a model of DIO. Body weight, blood fasting, total cholesterol, triglycerides and glucose levels, as well as fecal pellet frequency and stool water content, were evaluated the day before sacrifice. Epididymal fat weight and colonic malondialdehyde (MDA) concentration were then assessed. The expression and localization of $A_{2B}R$ and the neuronal marker HuC/D were determined by immunofluorescence. Colonic longitudinal muscle strips (LMS) were set up in organ baths with Krebs solution containing guanethidine and connected to isometric transducers. The effects of MRS-1754 (MRS, 0.01 μM; $A_{2B}R$ antagonist) were assayed on contractile responses elicited by electrical stimulation (ES; 0.5 ms, 28 V, 10 Hz), carbachol or substance P (SP) [both at 1 μM in the presence of tetrodotoxin]. DIO mice displayed altered blood metabolic indices, increments of body weight, epididymal fat weight and colonic MDA levels, as well as reduced fecal pellet frequency and decreased stool water content, as compared with RD mice. In DIO mice, the colonic distribution of $A_{2B}R$ immunoreactivity was significantly increased in myenteric HuC/D^+ neurons. MRS did not affect ES-induced contractions in LMS from RD mice, while it enhanced electrically evoked responses in colonic preparations from DIO mice (+32.1±3.5%). This enhancing effect was not affected upon blockade of nitrergic pathways with the *nitric oxide synthase* inhibitor L-N^w-nitroarginine methyl ester (L-NAME) [+35.6±2.8%]. By contrast, the enhancing effect of MRS was no longer observed on ES-induced cholinergic contractions evoked in the presence of L-NAME, L-732.138, GR159897 and SB218795 (NK_1, NK_2 and NK_3 receptor antagonists, respectively). Under ES-induced NK_1-mediated tachykininergic contractions (incubation with atropine, L-NAME, NK_2 and NK_3 antagonists), the increasing motor effect of MRS was still evident (+27.6±5.7%). MRS did not affect contractions elicited by carbachol or exogenous SP. In the model of HFD-induced obesity, metabolic alterations are associated with colonic oxidative stress and motor dysfunctions. In this setting, endogenous adenosine takes part to a significant inhibitory control on NK_1-mediated tachykininergic colonic contractions, which is likely mediated by an increased expression of $A_{2B}R$ at level of myenteric neurons.

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