ROLE OF ENTERIC GLIA IN THE DEVELOPMENT OF INTESTINAL DYSMOTILITY ASSOCIATED WITH HIGH FAT DIET IN MICE

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Introduction: Enteric glial cells (EGCs) play a critical role in the gut, modulating several physiologic activities as well as the onset and development of intestinal disorders. However, data on the involvement of EGCs in obese-related intestinal dysmotility are lacking. Therefore, this study examined the role of EGCs in colonic neuromuscular dysfunctions in a mouse model of high fat diet (HFD)-induced obesity.

Methods: C57BL/6mice were fed standard diet (SD; 18% calories from fat) or HFD (60% calories from fat) for 1, 2or 8weeks to obtain an obesity model. Body and epididymal fat weight, blood fasting metabolic parameters, as well as fecal pellet frequency and stool water content were evaluated the day before sacrifice. Colonic longitudinal muscle strips were set up in organ baths with Krebs solution, containing guanethidine, N^{ω}-nitro-L-arginine methylester (L-NAME), atropine, GR159897and SB218795to record tachykininergic NK1-mediated contractions, and connected to isometric transducers. The effects of fluorocitrate (FC, 50 mM; gliotoxin) were assayed on tachykininergic contractions evoked by electrical stimulation (0.5ms, 28V, 10Hz) or exogenous substance P (SP; in the presence of tetrodotoxin). Colonic interleukin (IL)-1β, IL-6and malondialdehyde (MDA) levels were measured by ELISA. The expression of occluding in colonic tissues was examined by western blot. Expression and localization of substance P, S100b and GFAP were assessed in whole mount specimens of colonic longitudinal muscle-myenteric plexus by immunohistochemistry. To mimic the exposure to HFD, cultured EGCs were incubated with palmitate (PA, 400 mM) and/or lipopolysaccharide (LPS, 10 mg/ml). SP and IL-1β levels were assayed in the culture medium by ELISA.

Results: After 1or 2weeks, HFD mice did not display alterations of body and epididymal fat weight when compared to SD mice. However, a marked increase in both parameters was observed after 8weeks of HFD. Alterations of blood cholesterol and triglyceride levels were observed with HFD starting from week 2, with an increase in blood glucose levels at week 8, as compared with SD. HFD mice showed a decrease in fecal pellet frequency and stool water content. In in vitro experiments, the electrically evoked tachykininergic contractions were significantly enhanced in mice fed with HFD for 2or 8weeks, as compared with SD animals. In this setting, the presence of FC in Krebs solution blunted the enhancement of contractions recorded in HFD mice. The contractions elicited by direct activation of NK1-tachykininergic receptors on longitudinal smooth muscle with SP were not significantly enhanced in HFD, neither affected by FC both in HFD and SD mice. At week 1, HFD mice displayed a reduction of colonic occludin expression, as compared with SD-mice; a further decrease was observed at week 2and week 8. Of note, HFD mice were characterized by an increase in colonic IL-1β and MDA levels at week 2, to a greater extent, at week 8, as compared with SD. Colonic IL-6levels as well as substance P and S100β density in myenteric ganglia of HFD mice were increased at week 8, but not at week 1or 2. In cultured EGCs, the incubation with LPS or palmitate alone did not exert any significant effect n both IL-1β and SP release, as compared with control cells. However, co-incubation with palmitate plus LPS led to a significant increase in both SP and IL-1β release.

Conclusions: The model of HFD-induced obesity is characterized by colonic dysmotility as well as by an increase in bowel inflammation and oxidative stress, occurring in parallel with enteric gliosis. In this setting, the hyperactivation of EGCs could take a significant part to the development of enteric motor disorders, through an increase in tachykininergic activity and release of pro-inflammatory mediators.