Enteric glia is involved in colonic dysmotility associated with high fat diet-induced obesity

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Introduction. Enteric glial cells (EGCs) are known to be involved in the regulation of bowel motility, as well as the onset and development of several digestive disorders. However, the putative role of EGCs in obesity-related intestinal motor dysfunctions is currently unknown. Therefore, the present study was performed in order to evaluate the involvement of EGCs in colonic neuromuscular alterations in a mouse model of diet-induced obesity.

Methods. C57BL/6 male mice were fed with standard diet (SD; 18% calories from fat) or high fat diet (HFD; 60% calories from fat) for 8 weeks to obtain a model of diet-induced obesity. Body and epididymal fat weight, blood fasting glucose levels, as well as fecal pellet expulsion frequency and stool water content were evaluated the day before sacrifice. Colonic longitudinal muscle strips (LMS) were set up in organ baths containing Krebs solution added with guanethidine (10 □M) and connected to isometric force transducers. The effects of the gliotoxin fluorocitrate (FC, 50 □M) were tested on contractile responses evoked by electrical stimulation (ES; 0.5 ms, 28 V, 10 Hz), carbachol or substance P (SP) (both 1 □M in the presence of tetrodotoxin). Colonic interleukin (IL)-1β, IL-6 and malondialdehyde (MDA) levels were assayed. Expression and localization of the enteric neuronal HuC/D and glial cell GFAP markers were assessed by immunofluorescence.

Results. Mice fed with HFD displayed increments of body weight, epididymal fat weight and blood glucose levels, as well as a decreased fecal pellet frequency and stool water content (Table 1). In in vitro experiments, the blockade of nitrergic pathways with L-N□-nitroarginine methyl ester (L-NAME) did not affect electrically induced contractions of LMS from SD mice, while it increased ES-induced contractions in colonic preparations from HFD mice (+34.8□3.7%). Moreover, colonic LMS from HFD mice were characterized by a significant increase in ES-induced NK1-mediated tachykininergic contractions [elicited upon incubation with atropine, L-NAME, GR159897 and SB218795 (NK2 and NK3 antagonists, respectively)] (+152□4%), when compared with SD animals. No differences were observed in carbachol- or exogenous SP-induced contractions in HFD mice as compared to SD animals. The increase in nitrergic and NK1-mediated tachykininergic motor responses were significantly blunted upon incubation of LMS with the gliotoxin FC. Carbachol- or exogenous SP-induced contractions were not affected by FC. Of note, HFD mice were characterized by an increase in colonic IL-1β, IL-6 and MDA levels, as well as by a significant increase in colonic GFAP immunoreactivity as compared with SD mice (Table 1).

Conclusions. The intake of HFD is characterized by development of colonic dysmotility as well as an increase in bowel inflammation and oxidative stress, in parallel with enteric gliosis. In this setting, hyperactivation of EGC appears to be critically involved in colonic dysmotility through an involvement of nitrergic and tachykininergic pathways.

Table 1. Effects of standard diet (SD) and high fat diet (HFD) (mean±SEM from 8 experiments; *P < 0.05, versus SD mice)

	SD	*HFD*
BODY WEIGHT GAIN	+30±3.5%	+78±4.2%*
EPIDIDYMAL FAT WEIGHT (g)	0.45±0.4 (1.5% of body weight)	1.8±0.2* (6% of body weight)
BLOOD GLUCOSE (mg/dl)	124±5	172±3 *
PELLETS FREQUENCY (1h)	13±1	5±2*
STOOL WATER CONTENT	65±3%	38±5%*
IL-1β (pg/mg total proteins)	3.8±0.5	8.2±0.7*
IL-6 (pg/ml)	0.12±0.01	0.46±0.04*
MDA (nmol/mg)	15±3.2	48±5*