Involvement of Catechol-O-MethylTransferase Genetic Reduction in Murine Intestinal Dysmotility: a Possible Link between Psychiatric Disorders and Irritable Bowel Syndrome

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Irritable bowel syndrome (IBS) is a still poorly understood functional disorder, characterized by abdominal discomfort, associated with different psychiatric symptoms (e.g. anxiety and depression) in the absence of organic disease (Karling et al., 2011; Grzesiak et al., 2014). Since psychiatric disorders and pain syndromes are associated with low catechol-O-methyltransferase (COMT) activity and these conditions are both related to IBS we assessed whether COMT genetic reduction affects enteric nervous system (ENS) homeostasis and intestinal function. Female COMT heterozygous ($COMT^{+/-}$) and wild-type ($COMT^{+/+}$) mice (12±2 weeks) were used and their genotypes were confirmed by PCR on mouse tail DNAs. To evaluate ileum integrity, histological analysis was performed on ileal specimens (5-10 cm from ileocecal valve) obtained from $COMT^{+/-}$ and WT mice. Gastrointestinal transit was assessed in vivo by measuring the distribution of nonabsorbable fluorescein isothiocyanate labeled dextran (FITC-dextran, 70 KDa) 30 minutes post intragastric administration (Brun et al., 2013). Contractile activity of isolated ileum segments, mounted vertically in organ baths, was evaluated as changes in muscle tension recorded by isometric transducers following carbachol (0.001-100 μM) or 60 mM KCl treatment, electric field stimulation (EFS, 1-50 Hz) or EFS-induced non-adrenergic non-cholinergic (NANC) inhibition (EFS=10 Hz, 1 μM atropine and 1 μM guanethidine, with and without 0.1 mM L-NAME). In ileum whole mount preparations immunoreactivity changes in neuronal markers, such as HuC/D and betaIII-tubulin, and in glia markers, such as S100beta and glial fibrillary acidic protein (GFAP) were determined by confocal immunofluorescence. Acetylcholinesterase and NADPH-diaphorase biochemical assays together with nNOS immunohistochemistry were performed to evaluate the integrity of ENS neurochemical code. GluN1 mRNA levels were also studied in LMMP preparations obtained from transgenic animals. Hematoxylin-eosin staining of ileal frozen sections showed a significant increase of muscle layer thickness (+87±6%, $p<0.05$) in $COMT^{+/-}$ mice compared with WT mice. COMT genetic reduction determined an altered gut distribution of FITC-dextran associated to a significant increase of gastrointestinal transit in $COMT^{+/-}$ compared to WT mice (GC 6.5±0.2 vs 5.7±0.2, respectively; $p<0.05$). In vitro contractility studies have shown altered receptor and not-receptor mediated responses (-41±8%, $p<0.01$ of Emax for carbachol and -38±5%, $p<0.01$ of contraction to KCl, respectively) together with a reduced neuronal cholinergic transmission (-33±6%, $p<0.01$ at 20 Hz) associated with an enhanced nitrergic inhibitory response (+114±11%, $p<0.05$ at 10 Hz) in $COMT^{+/-}$ mice. In myenteric ganglia of $COMT^{+/-}$ mice the ileal distribution of S100beta and GFAP immunoreactivity significantly increased. $COMT^{+/-}$ mice displayed a reduced staining of acetylcholinesterase$^+$ fibers (-26±3%, $p<0.01$) and NADPH-diaphorase$^+$ neurons (-23±4%, $p<0.01$) together with an altered distribution of nNOS immunopositive neurons. The mRNA levels of GluN1 increased by 1,7 fold in LMMPs obtained from transgenic mice compared to controls. Our study provides evidence that changes in catecholaminergic transmission due to genetic-driven COMT defective activity determine anomalies in ENS architecture, neurochemical coding and function leading to intestinal dysmotility. We cannot exclude that such changes may also affect the integrity and function of the central nervous system, via the brain-gut axis, being involved in the pathogenesis of IBS.

Brun et al. (2013). *Gastroenterology*. 145, 1323-33.
Grzesiak et al. (2014). *Adv Clin Exp Med.* 23, 987-92.
Karling et al. (2011). *PLoS One.* 6, e18035.