graft, since conduits need to be colonized, while in the autograft most of the players involved in the nerve regeneration are already on site. It has been previously shown by others that within the tissue spontaneously formed to reconnect injured nerves, called "nerve bridge", Schwann cells use endothelial cells as a path. To better understand what happens during regeneration inside a conduit, we investigated if Schwann cells behave the same way when they migrate within a conduit. To this aim, adult female rat median nerves were injured and repaired with a 10 mm chitosan conduit and the nerve portion regenerated within the conduit was analysed at different time points (7, 14, 21 and 28 days) by means of confocal immunofluorescence analysis of sequential thick slices. As hypothesized, our data show that migrating Schwann cells use newly regenerated blood vessels as a substrate for migration within the conduit. These results confirmed that, in this experimental paradigm, angiogenesis within nerve conduits plays a key role, not only to sustain cell survival, but also to provide a path for migrating repair Schwann cells, thus suggesting that factors promoting vascularization might be used to promote nerve regeneration within longer conduits.

BENZO[a]PYRENE ALTERS ELECTROPHYSIOLOGICAL PROPERTIES AND GnRH RELEASE IN hfHYPO CELLS

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Benzo[a]pyrene (BaP) is a widespread pollutant that can act as endocrine disrupting chemical (EDC) and interfere with reproductive function and embryo development. To date, the study of BaP effects on human reproductive axis at central level is lacking. The central regulatory network of the reproductive system is mediated by gonadotropin-releasing hormone (GnRH) neurons, which release in a pulsatile manner the GnRH into the hypothalamic-hypophyseal portal circulation and maintain the reproductive function. Here, we investigated the effects of BaP on GnRH neuron function taking advantage of a primary culture isolated from the human fetal hypothalamus (hfHypo). hfHypo cells express the enzymes cytochrome P450 (CYP1A1 and CYP1B1), required for metabolic activation of BaP and that expression was strongly induced by Bap exposure (10 μM, 24 h). From a functional point of view, BaP exposure significantly reduced the mRNA level of the kisspeptin receptor (KISS1R), the main physiological regulator of GnRH neuron function. Interestingly, BaP increased phospho-ERK1/2 signaling which is a known intracellular mechanism associated with KISS1R by Kisspeptin activation. Moreover, BaP induced changes in electrophysiological membrane properties causing a significant depolarizing effect and significantly increased GnRH secretion, with both effects being not changed by the addiction of kisspeptin. In conclusion, our findings demonstrate that BaP may affect GnRH neuron function by altering electrophysiological properties and interfering with KISS1R signalling and GnRH secretion, suggesting a possible EDCs-related mechanism at central level underlying reproductive function alterations.

NOVEL FINDINGS ON GENETICALLY DRIVEN ENTERIC NEUROPATHY: THE RAD21 KNOCK-IN MOUSE MODEL

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RAD21 is a double-strand-break repair protein of the cohesin complex and a regulator of transcription processes, which plays key roles in the maintenance and survival of various cell types including neurons. In a consanguineous family with a clinical phenotype of neurogenic chronic intestinal pseudo-obstruction, *i.e.* the worst expression of gut dysmotility, our group identified a novel causative RAD21 (Ala622Thr) missense mutation. By immunohistochemistry, we showed Rad21 immunoreactivity (IR) in a subset of neurons of the mouse enteric nervous system. Furthermore, we developed a genetically re-constructed Rad21 conditional knock-in (Rad21KI) mouse carrying the Ala626Thr mutation (mouse homolog of human mutation) to understand how the RAD21 mutation impairs gut motility. The aim of this study was to perform a qualitative and quantitative characterization of myenteric neurons in the small intestine (duodenum, jejunum and ileum) and colon of Rad21KI *vs.* wild type (WT) mice. Immunohistochemical analysis was performed in whole mount myenteric plexus preparations using the pan-neuronal marker HuC/D, choline acetyltransferase (ChAT, a cholinergic marker for excitatory motor neurons) and neuronal nitric oxide synthase (nNOS, a nitrergic marker for inhibitory motor neurons). The total number of HuC/D myenteric neurons did not significantly change in Rad21KI *vs.* WT in the small intestine and colon. However, in the small intestine, we showed that Rad21KI HuC/D/ChAT-IR myenteric neurons/field were 18.43±1.6 *vs.* 31.18±2.4 of WT mice ($P \leq 0.005$); HuC/D/nNOS-IR myenteric neurons/field were 11.87±1 in Rad21KI *vs.*14.83±0.6 in WT ($P \leq 0.005$). HuC/D/ChAT-IR myenteric neurons/field in the mouse colon were 19.88±2.1 in Rad21KI *vs.* 32.15±3.8 in WT mice ($P \leq 0.005$). There were no significant changes to HuC/D/nNOS-IR myenteric neurons in the colon of Rad21KI and WT mice.

In conclusion, the total number of HuC/D neurons showed no changes in both Rad21KI and WT mice. However, the small intestine of Rad21KI mice showed a significant decrease of cholinergic and nitrergic neurons. Conversely, in the colon, only cholinergic neurons were reduced. Our findings provide an accurate neurochemical basis to understand the neuropathic features of the RAD21-related CIPO patients. Further analyses are needed to decipher the mechanisms through which individual subsets of nitrergic and cholinergic myenteric neurons are affected in distinct gut segments of the Rad21KI mouse.