Volume 50(3-4):159, 2006
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

DISSERTATION SUMMARY

The effect of chronic alcohol consumption on nitric oxide synthesis in the murine and rat gastrointestinal tract

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In the gastrointestinal tract, chronic alcohol consumption disturbs normal intestinal motor function leading to motility disorders.

Nitric oxide (NO) is the major mediator of inhibitory neurotransmission in the gut. NO is synthesized by three distinct isoform of NO synthase: neuronal (nNOS), endothelial (eNOS) and inducible (iNOS) NO synthase through distinct regulatory mechanisms. nNOS-immunoreactive nerve cells in the gastrointestinal tract have been localized to a subpopulation of neurons in the myenteric plexus.

The role of nNOS-derived NO in the physiological relaxations of murine gastrointestinal muscle has been demonstrated in pharmacological studies and in knockout animals. Inhibitors of nNOS have been shown to reduce nerve-mediated relaxations. However, the involvement and the regulation of NO synthesis under pathophysiological conditions are unclear.

We hypothesized the role of myenteric neuronal NO in the observed motility disturbances following chronic alcohol consumption. To test this, we have quantified the total number of myenteric neurons and nNOS-immunoreactive neurons in the mouse jejunum and in all segments of the rat intestine. We measured the release of NO in real time with a NO-reactive fluorescent dye and the effect of chronic alcohol consumption *in vitro* on nitrergic relaxations to electrical field stimulus (EFS) and exogenous NO in the mouse jejunum. Furthermore, NOS activity and protein content was determined in all segments of the rat intestine.

Mice received a gradually increasing concentration of ethanol in water with a final concentration of 20% for five weeks, controls received isocaloric sucrose solution or water. Rats received either 20% aqueous ethanol solution or water for 8 weeks**.** Using PGP9.5- and nNOS-immunostaining, the proportion of nNOS-immunopositive myenteric neurons was assessed. NO production was visualized and measured by confocal microscopy on tissue loaded with the fluorescent dye DAF-FM. The effect of ethanol treatment on nitrergic relaxations to EFS and exogenous NO of jejunal circular muscle strips was investigated *in vitro*. Small intestinal transit was measured *in vivo* after intragastric gavage of Evans blue. NOS activity in rat intestinal segments was measured by the conversion of [^{3}H]L-citrulline from [^{3}H]L-arginine and protein content was determined by Western blotting.

The percentage of nNOS-immunoreactive neurons decreased significantly after chronic alcohol consumption compared to controls, while the total number of neurons did not change in both mice and rat samples. DAF-FM fluorescence was significantly increased in neurons after chronic alcohol consumption and only partial colocalization with nNOS was observed. In jejunal circular muscle preparations, the nitrergic nerve-mediated relaxations to 1, 2 and 4 Hz EFS significantly decreased after ethanol treatment compared to controls, while relaxations to exogenous NO remained unchanged. Small intestinal transit was significantly delayed in mice after chronic alcohol consumption when compared to water control mice but significantly accelerated when compared to the sucrose control group.

Constitutive NOS activity significantly decreased after chronic ethanol treatment in the jejunum, ileum and colon but not in the duodenum. Inducible NOS activity levels were not significantly affected by ethanol. The nNOS protein content measured by Western blotting indicated a significant decrease in the colon after ethanol consumption, while in other intestinal segments change was not detectable.

Our results suggest an adaptive and region-dependent change in the synthesis of NO most particularly by the neuronal isoform of NOS in response to chronic alcohol consumption. The reduction of its activity and protein content as well as the reduction in nNOS-immunopositive cell number indicates a downregulation of the enzyme. The impairment of nNOS might partially account for the observed motility disturbances.

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