Nicotinic receptors and spleen implication in TNBS-induced colitis in mice

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Background and aim: Increasing evidence indicates that vagus nerve signalling can dampen inflammation through the activation of nicotinic receptors (*nAChRs*) in different clinical and experimental conditions, like Inflammatory Bowel Disease (IBD) (1), and such '*Cholinergic Anti-inflammatory Pathway*' (CAP) remains to be elucidated in terms of nAchRs subtypes and spleen involvement (2).
Our aim was to pharmacologically investigate the role played by Î±7 and Î±4β2 *nAChRs* in the modulation of inflammatory responses induced in 2,4,6-Trinitrobenzene Sulfonic Acid (TNBS) colitis, a murine IBD model, by administering selective Î±7 and Î±4β2 ligands in normal and splenectomized mice.

Methods: Colitis was induced in female Swiss mice (7-11 weeks old) by enema (i.r.) administration of 5mg/mouse TNBS, 6 days after skin sensitization. Mice were randomly assigned to:

CTR: saline (0.9% NaCl) 10 mL/kg
AR-R: Î±7 agonist AR-R17779 1.5 mg/kg
MLA: Î±7 antagonist MethylLycAconitine 1 mg/kg
TC: Î±4β2 agonist TC2403 5 mg/kg
DBE: Î±4β2 antagonist Dihydro-βErythroidine 1.5 mg/kg

Pharmacological treatments started 8h after TNBS enema and were administered subcutaneously (s.c.) twice daily for 3 days. Normal mice (**SHAM**) received saline 50 µL i.r. and 10 mL/kg s.c..

In a second series of experiments, mice splenectomized (**SPX**) 14 days before colitis induction, were administered with saline (**SPX-CTR**) or with AR-R17779 1.5mg/kg (**SPX-AR**). We determined Disease Activity Index (DAI) to assess clinical outcome, colonic Macroscopic Score (MS) and thickness to evaluate local damage, colonic and lung myeloperoxidase (MPO) activity as marker of local and systemic granulocyte infiltration. Splenic and mesenteric lymph nodes $CD3^+$ T cells were counted by flow cytometry. All animal experiments were performed according to the guidelines for the use and care of laboratory animals (DL 26/2014).

Results: CTR mice, compared to **SHAM**, showed markedly higher DAI ($P<0.001$), MS ($p<0.001$), colonic thickness ($p<0.01$), colonic ($P<0.001$) and lung MPO ($p<0.01$); $CD3^+$ T cells were increased in the spleen ($P<0.01$) and slightly decreased in mesenteric lymph nodes.
TC, with respect to **CTR**, improved only DAI ($p<0.01$), while **DBE** significantly reduced colonic MPO and DAI ($p<0.05$). **AR-R** remarkably decreased MS, colonic thickness ($p<0.01$) and MPO activity ($p<0.05$), while **MLA** slightly augmented MS and lung MPO but did not affect the other markers. In **SPX-AR** mice, Î±7 agonist reduced colonic thickness ($p<0.01$), compared to **SPX-CTR**, but, on the other hand, it worsened DAI and lost efficacy in decreasing MS and colonic MPO; no effects were produced on T cells, either with or without SPX.

Conclusions: Either **TC** or **DBE** evoked weak and seemingly contradictory effects, thus suggesting a role of Î±4β2 *nAChR* subtype in TNBS-induced colitis that deserves further investigations.
Pharmacological activation of Î±7 *nAChRs* showed beneficial effects not completely prevented by splenectomy allowing us to speculate that the anti-inflammatory activity of stimulated Î±7 *nAChRs* is not exclusively spleen mediated.

References:

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