The Journal of Physiology

Statistical Summary Document

Manuscript Title: Extracellular metabolism of the enteric inhibitory neurotransmitter β-nicotinamide adenine dinucleotide (β-NAD) in the murine colon

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Animal model used, if applicable: C57BL/6 mice (Wildtype, WT), Cd38-KO mice, Nt5e-KO mice, Cd38-KO/ Nt5e-KO knockout mice, Enpp1-KO mice, FACS-purified SMCs, ICC, and PDGFRα+ cells

Underlying hypothesis:
Hypothesis 1: The enteric neurotransmitter β-NAD is metabolized to ADPR by CD38, to AMP by ENPP1, and to adenosine (ADO) by NT5E in the mouse colon muscularis.
Hypothesis 2: β-NAD metabolizing enzyme activities are differentially distributed in smooth muscle cells (SMC), interstitial cells of Cajal (ICC), and cells expressing platelet-derived growth factor receptor α (PDGFRα+ cells) in the mouse colon muscularis.

Definitions of 'n': [Define 'n'. If definitions differ, please indicate which definition applies to which experimental question number.]
'n' = number of observations
One observation is made per one superfusion chamber. In experiments with colon muscularis (Q1-6) each superfusion chamber contained colon tissue preparations from 2 mice. In experiments with FACS-purified cells (Q7-11) each superfusion chamber contained 35,000-62,000 cells isolated from 4-5 mouse colons.

All data are expressed as Mean ± SD. The actual values of mean, SD, and P are found in Results in main text.

Experimental questions:
Q1: Does tunica muscularis of the murine colon exhibit the machinery for NAD degradation to ADPR, AMP and ADO?
Q2: Does deletion of *Cd38* inhibit the formation of eADPR, eAMP and eADO from eNAD in the murine colon muscularis?
Q3: Does deletion of *Enpp1* inhibit the formation of eAMP and eADO from eNAD in the murine colon muscularis?
Q4: Does deletion of *Nt5e* inhibit the degradation of the eNAD product eAMP to eADO?
Q5: Does deletion of both *Cd38* and *Nt5e* result in inhibited formation of eADPR and eADO from eNAD and in accumulation of eAMP?
Q6: What roles do CD38, NT5E and ENPP play in the NAD metabolism in the murine colon tunica muscularis?
Q7: Can eNAD metabolism to eADPR, eAMP and eADO be detected in freshly dispersed cells of murine colon muscularis?
Q8: Are SMCs in mouse colon muscularis equipped to metabolize NAD to ADPR, AMP and adenosine?
Q9: Are ICC in mouse colon muscularis equipped to metabolize NAD to ADPR, AMP and adenosine?
Q10: Are PDGFRα+ cells in mouse colon muscularis equipped to metabolize NAD to ADPR, AMP and adenosine?
Q11: Do SMCs, ICC and PDGFRα+ cells of colon muscularis have similar capacity to metabolize NAD?

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Statistical summary table:

Experimental question number*	Finding/ conclusion	Experimental location/ variable e.g. cortex vs cerebellum or genotype	Mean value (or other summary statistic)	SD	n (value)	P**	Units	Data comparisons e.g. WT vs KO	Statistical test	Any other variable e.g. subjects' age or sex	Figure/table in which data are presented	Comments e.g. observation
1.	eNAD is degraded to eADPR, eAMP and eADO in colon tissue of WT mice.	WT colon vs. no tissue	-	-	22 (-tissue) 12 (+tissue)	-	fmol/mg tissue	eNAD (-tissue) vs. eNAD (+tissue, WT)	One-way ANOVA with Dunnett's multiple comparison test (GraphPad Prism, v. 8.3.0)		Fig. 2	Data for substrate in no contact with tissue (- tissue) is common for WT and KO tissue preparations (n = 22) and is used in multiple comparison analyses (Q1-6).
2	CD38 mediates the metabolism of NAD to ADPR; NAD can also be degraded to AMP by an alternative pathway that	*Cd38*-KO colon vs. no tissue	-	-	6	-	fmol/mg tissue	eNAD (-tissue) vs. eNAD (+tissue, WT vs. *Cd38*-KO)	One-way ANOVA with Dunnett's multiple comparison test		Fig. 2	In the absence of *Cd38*, eNAD failed to form eADPR, but produced eAMP and eADO.

	does not involve CD38.											
3	ENPP1 mediates the formation of AMP from either ADPR or NAD.	*Enpp1*-KO colon vs. no tissue	-	-	3	-	fmol/mg tissue	eNAD (-tissue) vs. eNAD (+tissue, WT vs. *Enpp1*-KO)	One-way ANOVA with Dunnett's multiple comparison test		Fig. 2	Deletion of Enpp1 resulted in accumulation of eADPR and decreased formation of eAMP and eADO from eNAD.
4	NT5E mediates the degradation of eAMP to eADO in colon muscularis.	*Nt5e*-KO colon vs. no tissue	-	-	12	-	fmol/mg tissue	eNAD (-tissue) vs. eNAD (+tissue, WT vs. *Nt5e*-KO)	One-way ANOVA with Dunnett's multiple comparison test		Fig. 2	In colon muscularis from Nt5e-KO mice, eNAD metabolism resulted in increased amounts of eAMP and decreased formation of eADO when compared with WT controls.
5	In colon muscularis, eNAD is degraded to ADPR by CD38 and eAMP is degraded to eADO by Nt5E.	*Cd38*-KO/*Nt5e*-KO colon vs. no tissue	-	-	8	-	fmol/mg tissue	eNAD (-tissue) vs. eNAD (+tissue, WT vs. double-KO)	One-way ANOVA with Dunnett's multiple comparison test		Fig. 2	In colon muscularis lacking both CD38 and Nt5e, the degradation of eNAD resulted in reduced formation of eADPR and eADO, but increased amounts of eAMP.

6	In colon muscularis, eNAD is degraded to ADPR by CD38, to AMP by Enpp1, and to eAMP by NT5E.	Tissues of WT, Cd38-KO, Enpp1-KO, Nt5e-KO, Cd38-KO/Nt5e-KO mouse colons vs. no tissue	-	-	22, 12, 6, 3, 12, 8	-	fmol/mg tissue	eNAD (-tissue) vs. eNAD (+tissue, WT vs. *Cd38*-KO vs. *Nt5e*-KO vs. *Enpp1*-KO vs. DKO)	One-way ANOVA with Dunnett's multiple comparison test		Fig. 2	Observations described in Q1-5.
7	eNAD metabolism to eADPR, eAMP, and eADO can be successfully detected in freshly dispersed cells from colon muscularis.	Cells vs. no cells	-	-	12	-	fmol/1000 cells	eNAD (-cells) vs. eNAD (+cells)	Paired, two-tailed t-test		Fig. 4	Superfusion of freshly dispersed cells with eNAD resulted in the appearance of the eNAD products eADPR and eADO.
8	SMCs do not express CD38, but express high activity of NT5E.	Cells vs. no cells	-	-	4	-	fmol/1000 cells	eNAD (-cells) vs. eNAD (+cells)	One-way ANOVA with Dunnett's multiple comparison test		Fig. 5	Superfusion of purified SMCs with eNAD did not result in formation of eADPR, but produced significant amounts of eADO.

9	ICCs are not equipped to metabolize NAD.	Cells vs. no cells	-	-	4	-	fmol/1000 cells	eNAD (-cells) vs. eNAD (+cells)	One-way ANOVA with Dunnett's multiple comparison test		Fig. 5	Superfusion of purified ICC with eNAD resulted in no formation of eADPR or eADO.
10	PDGFRα+ cells do not express CD38, but express NT5E.	Cells vs. no cells	-	-	4	-	fmol/1000 cells	eNAD (-cells) vs. eNAD (+cells)	One-way ANOVA with Dunnett's multiple comparison test		Fig. 5	Superfusion of PDGFRα+ cells with eNAD resulted in no formation of eADPR and in production of eADO.
11	SMCs, ICC, and PDGFRα+ cells play differential roles in the extracellular metabolism of NAD in the murine colon.	SMCs vs. ICC. vs. PDGFRα+ cells vs. no cells	-	-	12, 4, 4, 4	-	fmol/1000 cells	eNAD (-cells) vs. eNAD (+SMCs vs. +ICC vs. +PDGFRα+ cells)	One-way ANOVA with Dunnett's multiple comparison test		Fig. 5	SMCs, ICC, and PDGFRα+ cells do not express CD38. SMCs express high amounts of Nt5e, PDGFRα+ cells express moderate amounts of Nt5e, and ICC lack Nt5e activity.

*You may use multiple lines for the same question to indicate multiple comparisons

** Authors may wish to make the text bold where p is considered significant against a stated confidence limit