Response to Mutafova-Yambolieva and Sanders

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TO THE EDITOR: My research team and I appreciate the thoughts of Dr. Violetta Mutafova-Yambolieva and Prof. Kenton M. Sanders (VMY-KMS; Ref. 14a) to enhance awareness for our recent paper on β-nicotinamide adenine dinucleotide (β-NAD) (17). We agree fully with VMY-KMS that roles for purinergic neurotransmission in the gastrointestinal tract are multiple and can be controversial.

Major findings in our study, questioned by VMY-KMS, were twofold. First was failure to find supporting evidence for the VMY-KMS hypothesis that β-NAD is a physiological ligand for receptors expressed by guinea pig or human small intestinal smooth muscle and therefore not likely to have a role as an inhibitory neurotransmitter released by enteric inhibitory musculomotor neurons at intestinal neuromuscular junctions (5, 11, 13, 14). Our findings and conclusions in this respect mirrored those reported by Gallego et al., whose methods were essentially the same as ours (8, 9, 12). VMY-KMS suggest that there are errors in our interpretation of the data and, by inference, errors also in the data of Gallego et al. Gallego et al. found for human colon and we found for human small intestine that β-NAD was much less potent than ATP or MRS2365 as a hyperpolarizing agonist. High concentrations of β-NAD were needed to evoke miniscule responses and the action was not blocked by selective P2Y1 purinergic receptor antagonists. Moreover, exogenously applied β-NAD evoked only small hyperpolarizing responses that did not replicate electrically evoked inhibitory junction potentials recorded during impalements of intestinal smooth muscle fibers with “sharp” microelectrodes.

Our second major finding was that β-NAD binds to adenosine A1 receptors expressed by motor nerve terminals at neuromuscular junctions in guinea pig colon and in human jejunum. VMY-KMS questioned validity of this finding. On the basis of our data, we concluded that activation of prejunctional adenosine A1 receptors by β-NAD suppressed release of inhibitory and excitatory musculomotor neurotransmitters and thereby reduced the amplitude of electrically evoked inhibitory junction potentials and twitchlike muscle contractions.

We are unsure of the rationale that provoked the concern of VMY-KMS about β-NAD action at adenosine A1 receptors, because β-NAD behaved according to classical adenosine A1 receptor pharmacology and caused effects expected for an agent that acts prejunctionally to suppress neurotransmitter release. In fact, workers in the Wood group established adenosine A1 receptor-mediated presynaptic inhibition as a significant neurophysiological event in the enteric nervous system in the early 90s (3, 4).

We can understand why VMY-KMS, in their letter, emphasized a preference for “picospritzing” as an experimental method and their concern about our interpretation of data obtained with classical organ bath pharmacology. The Wood group, when working at the University of Nevada and later at The Ohio State University, began publishing on use of micropressure and iontophoretic application of agents and transmitters from fine-tipped pipettes in studies of the enteric nervous system in the 1970s and continue to rely on it at present (10, 15, 19). Still, both methodologies have limitations. Application of putative neurotransmitters with the micropressure ejection method can be adjusted for short transient exposures that simulate functional transmitter release at neuronal synapses or neuroeffector junctions and thereby avoid problems associated with prolonged exposure and tachyphylaxis. The duration of the “puffs” can be manipulated to obtain pseudo concentration-response data. On the other hand, organ bath pharmacology yields valid analyzable data on concentration-response relations and receptor binding kinetics, not obtainable with picospritzing.

VMY-KMS state “We reported that brief contact of β-NAD with GI muscles (e.g., 1–5 s) causes formation of several metabolites, including adenosine. Thus effects attributed to A1 receptors by Wang et al. . . .1 may have been caused by adenosine generated by metabolism of β-NAD.” Moreover, VMY-KMS state “Loss of β-NAD effects, as described in Wang and coworkers . . . and in other pharmacological studies employing bath application of drugs . . . , is likely to represent deactivation of β-NAD through metabolism.”

We assume that by “loss of β-NAD effects” VMY-KMS are referring to failure of Gallego et al. (8, 9, 12) and ourselves to confirm an inhibitory neurotransmitter role for β-NAD at intestinal neuromuscular junctions, as hypothesized by VMY-KMS. We did not report, in our paper, any loss of the adenosine A1 receptor action of β-NAD on neuromuscular transmission during prolonged exposure to β-NAD in the bathing medium. Metabolic deactivation of β-NAD, as suggested by VMY-KMS, is therefore an unlikely explanation for our failure to validate the β-NAD hypothesis for inhibitory neurotransmission at intestinal neuromuscular junctions.

Adenosine is formed by action of ectonucleotidases following release of ATP at enteric synapses, neuromuscular junctions, and neuroglandular junctions, as pointed out by VMY-KMS and taken into account by the Ohio State Group in the past (6, 7, 10). In our experience, adenosine accumulates in the tissue bath and acts at presynaptic adenosine A1 receptors to suppresses synaptic transmission in the enteric nervous system, as we published years ago (3, 4). However, there is a possibility that some of the adenosine-like actions of β-NAD, in our study, could have resulted from partial enzymatic catabolism of β-NAD.

1 Reference citations in quotes from Mutafova-Yambolieva’s and Sanders’s letter (14a) have been deleted for clarity.

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The penultimate paragraph of the VMY-KMS letter states “Release of β-NAD was blocked by neurotoxins (TTX and ω-conotoxin GVIA) but release of ATP was unaffected, suggesting that ATP is most likely released from ganglionic sources but not motor neurons...” This observation, published by VMY-KMS, has little relevance for a discussion of inhibitory purinergic neuromuscular transmission in the intestine. This is the case because strong evidence supports release of ATP as a neurotransmitter at synapses in the enteric nervous system where postsynaptic excitation is mediated by P2Y1 receptors (10). Likewise, postganglionic sympathetic fibers, which project into enteric ganglia and synapse with enteric neurons, release ATP as a cotransmitter with norepinephrine (1, 2). In addition to neuromuscular junctions, enteric motor neurons, with cell bodies in the myenteric and submucosal plexuses, release ATP as an excitatory neurotransmitter at junctions with epithelial glands that secrete Cl\(^{-}\) and H\(_2\)CO\(_3\)^{−}, as well as neuromuscular junctions (6, 7, 16).

Finally, we wish to point out that we have examined effects of organ bath application of β-NAD on synaptic transmission in the enteric nervous system and found that it acts, much like adenosine, at inhibitory presynaptic adenosine A1 receptors to suppress the amplitude of fast nicotinic excitatory postsynaptic potentials and slow excitatory postsynaptic potentials in the same manner as for suppression of intestinal inhibitory junction potentials (18).

Overall, our data suggest that β-NAD behaves like an agonist at the adenosine A1 receptor subtype.

We wish to thank the editor for the opportunity to respond to the concerns expressed in the VMY-KMS letter about shortcomings in our paper.

**GRANTS**

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**REFERENCES**