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Commentary

D-Amino Acid Oxidase Inhibition: A New Glutamate Twist for Clozapine Augmentation in Schizophrenia?

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Despite clozapine, many patients with schizophrenia remain treatment resistant. Unfortunately, after many clinical trials with a diverse range of agents, there remain no evidence-based clozapine augmentation strategies. Against this background, the positive results of the randomized, double-blind, placebo-controlled trial of adjunctive sodium benzoate reported by Lin *et al.* (1) in this issue of *Biological Psychiatry* are extremely welcome, and they complement their earlier trial showing similar benefits in patients taking other antipsychotic medications (2). Equally, history teaches us to interpret results in this field cautiously and to consider carefully the evidence for efficacy as well as the hypothesized mechanism of action.

Lin *et al.* (1) studied 60 Taiwanese patients with schizophrenia who had persistent symptoms despite taking clozapine. The patients were randomized to groups with the addition of sodium benzoate (1 or 2 g/day) or placebo. The patients were more severely and chronically ill than in many comparable studies, and all were inpatients. The latter fact likely contributed to the remarkable 98% completion rate for the 6-week trial. There were four primary outcomes: Positive and Negative Syndrome Scale (PANSS) total score, Scale for the Assessment of Negative Symptoms, Global Assessment of Functioning, and Quality of Life Scale. Secondary outcomes included PANSS-positive and -negative symptom scores, Hamilton Depression Rating Scale, and a range of cognitive measures.

Both doses of sodium benzoate were more effective than placebo on the Scale for the Assessment of Negative Symptoms (effect size as measured by Cohen's $d = 0.65$ and 0.72). The higher dose was also effective for PANSS total score ($d = 0.80$), Quality of Life Scale ($d = 0.82$) and PANSS-positive scale ($d = 0.86$). All other outcomes, including Global Assessment of Functioning and cognition, did not differ between groups. Sodium benzoate was well tolerated, with no extrapyramidal side effects and no impact on neutrophil count.

The authors conclude, justifiably, that the trial shows a beneficial adjunctive effect of sodium benzoate on positive and negative symptoms of schizophrenia in patients taking clozapine. Appropriately, they also note several caveats, of which two are most important. After correction for having four primary outcomes and five secondary outcomes, significant effects of sodium benzoate were limited to the 2 g/day dose, with improvements on the PANSS (total and positive scores) and Quality of Life Scale. Also, although the effect sizes are moderate to large, the improvement in terms of points on the rating scales is much less impressive (e.g., a 4-point reduction on PANSS total score compared with placebo), and hence of questionable clinical relevance. The modest extent of the benefits is emphasized by the lack of change in Global Assessment of Functioning and the fact that negative symptoms as assessed by the PANSS negative scale did not change significantly despite the reduction on the Scale for the Assessment of Negative Symptoms. On the other hand, any statistically robust improvement in this refractory group of patients is encouraging, and it is possible that greater benefits might accrue from longer treatments, larger doses, or if administered with concurrent psychosocial or procognitive interventions.

Sodium benzoate is a food preservative and, at first sight, an unlikely treatment for schizophrenia. However, it was discovered many years ago to be an inhibitor of the enzyme D-amino acid oxidase (DAAO, also abbreviated as DAO), and this forms the basis for its use, according to the following rationale (3). DAAO metabolizes the amino acid D-serine, which is the major endogenous co-agonist of the synaptic *N*-methyl-D-aspartate (NMDA) subtype of ionotropic glutamate receptor (4). In schizophrenia, DAAO expression and enzyme activity is increased, and D-serine levels may be decreased (3,5). Reducing DAAO activity with sodium benzoate increases D-serine availability and thence enhances NMDA receptor (NMDAR) function. Parenthetically, DAAO was a prominent candidate gene for schizophrenia, but the locus has not been supported by genome-wide association studies.

Despite its attractiveness, each step in this hypothesized sequence of events linking sodium benzoate to NMDAR signaling is open to question. Establishing the potential value of sodium benzoate (or other DAAO inhibitors) in schizophrenia will benefit from careful examination of these issues.

First, it is not certain that sodium benzoate is acting via DAAO inhibition. Although Lin *et al.* (1) measured blood DAAO levels, they did not measure its activity, and circulating D-serine levels did not change in response to either dose of sodium benzoate. Moreover, preclinical data are varied regarding the ability of oral sodium benzoate to impact DAAO activity and D-serine levels in the brain. It therefore remains possible that sodium benzoate is acting independently of DAAO.

Second, assuming inhibition of DAAO activity is the basis for the therapeutic benefits, where is this occurring? Presumably this is in the brain, although the high levels of DAAO in the kidney and some other peripheral tissues should be noted. Within the brain, DAAO is conventionally considered to be limited to the cerebellum, to be expressed in astrocytes but not

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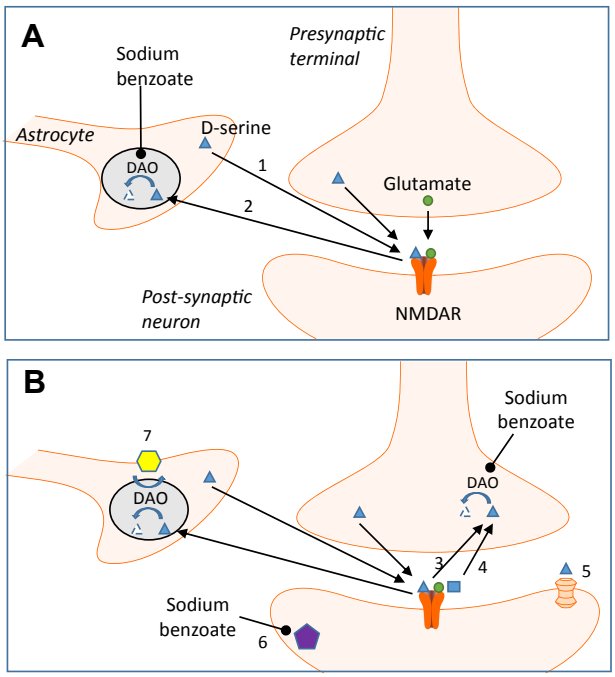

Figure 1. D-amino acid oxidase (DAAO), D-serine, and the *N*-methyl-D-aspartate receptor (NMDAR). **(A)** Conventional view. 1) D-serine (triangle) is released by astrocytes, and possibly by neurons, into the synaptic cleft where it binds to the synaptic NMDAR and acts as the co-agonist to accompany the agonist glutamate (circle). 2) D-serine is removed from the synapse into glia, where it is degraded by DAAO localized within peroxisomes (ellipse). In this simple model, sodium benzoate is presumed to act therapeutically by inhibiting astrocytic DAAO, leading to increased D-serine availability at the NMDAR. For clarity, the panel does not show the other major NMDAR co-agonist, glycine, which is regulated via separate pathways and mechanisms (and which itself has been targeted in schizophrenia). **(B)** Additional pathways that may be relevant to the therapeutic efficacy of sodium benzoate in schizophrenia. 3) DAAO is present in some neurons and concentrated at synaptic terminals. 4) DAAO metabolizes a range of substrates, including D-alanine (square), which is also an NMDAR co-agonist. 5) D-serine has actions beyond simply being an NMDAR co-agonist. 6) Sodium benzoate has effects other than DAAO inhibition. 7) DAAO has roles in addition to D-amino acid metabolism (hexagon). Steps 3–7 are not mutually exclusive, nor comprehensive, and their importance in the human brain in vivo is not well established. However, they illustrate some of the ways by which the therapeutic benefits of sodium benzoate reported by Lin *et al.* (1) may be mediated. For additional discussion, see the text and Verrall *et al.* (3).

neurons, and to be localized to peroxisomes (organelles involved in beta-oxidation and other catabolic reactions) (Figure 1A). None of these distributional properties would normally be associated with antipsychotic effects and have led to some understandable skepticism about the potential value of DAAO inhibition for schizophrenia. However, recent findings suggest a more "schizophrenia-relevant" profile of DAAO (Figure 1B). Thus, DAAO is in fact expressed in many brain regions, including the hippocampus and frontal cortex (6) and midbrain, wherein it modulates dopamine release (7). The latter functionality is notable because in contrast to its widespread expression in the brain, detectable DAAO activity is extremely low outside the cerebellum—a dissociation that remains unexplained. At the cellular level, DAAO messenger RNA and protein are found in neurons, not just in glia; moreover, within neurons, DAAO is localized to the presynaptic zone, much better placed to influence D-serine levels in the vicinity of synaptic NMDARs than it would be if DAAO were present exclusively in glial peroxisomes.

Third, what is the relevant DAAO substrate? DAAO metabolizes other D-amino acids than just D-serine, including D-alanine, itself an NMDAR co-agonist. Inhibition of these other substrates might help explain why sodium benzoate appears to have greater therapeutic efficacy in schizophrenia than is observed with D-serine administration. Indeed, the efficacy of sodium benzoate seen by Lin *et al.* (1) contrasts with the absent or even deleterious effects seen when augmenting clozapine with other NMDAR-enhancing strategies (e.g., the addition of D-serine or glycine).

Fourth, are the benefits of DAAO inhibition mediated solely by increasing NMDAR co-agonist availability? As examples of alternative possibilities, D-serine is also the agonist at the Gluδ2 receptor; and DAAO inhibition may affect oxidative processes, as suggested by the fact that Lin *et al.* (1) found an increase in catalase activity in the 2 g/day sodium benzoate treatment group.

Adding to these complexities, there are species differences in some properties of DAAO that hinder translation from preclinical pharmacological studies and the interpretation of findings in DAAO knockout mice. A specific issue concerns the possible regulation of DAAO by a gene called G72 (also known as DAAO activator), which is primate specific and itself shows some evidence of genetic association with schizophrenia (8).

Overall, while a positive NMDAR modulatory action remains the most likely explanation for the reported therapeutic effects of sodium benzoate, much remains to be determined, and additional mechanisms may exist. Having noted the many uncertainties, the positive findings of Lin *et al.* (1) argue for larger, longer, and more mechanistic clinical trials of sodium benzoate in schizophrenia. The results also give impetus to further studies of the neurobiology and pharmacology of DAAO, and to the development of DAAO inhibitors with greater potency and selectivity than sodium benzoate (9) and that can avoid the potential toxicity associated with the long-term, high-dose use of sodium benzoate (10).

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References

1. Lin C-H, Lin C-H, Chang Y-C, Huang Y-J, Chen P-W, Yang H-T, Lane H-Y (2018): Sodium benzoate, a D-amino acid oxidase inhibitor, added to clozapine for the treatment of schizophrenia: A randomized, double-blind, placebo-controlled trial. Biol Psychiatry 84:422–432.
2. Lane HY, Lin CH, Green MF, Hellemann G, Huang CG, Chen PW, *et al.* (2013): Add-on treatment of benzoate for schizophrenia: A randomized, double-blind, placebo-controlled trial of D-amino acid oxidase inhibitor. JAMA Psychiatry 70:1267–1275.
3. Verrall LJ, Betts JF, Burnet PWJ, Harrison PJ (2010): The neurobiology of D-amino acid oxidase (DAO) and its involvement in schizophrenia. Mol Psychiatry 15:122–137.
4. Papouin T, Ladapeche L, Ruel J, Saachi S, Labasque M, Hanini M, *et al.* (2012): Synaptic and extrasynaptic NMDA receptors are gated by different endogenous coagonists. Cell 150:633–646.
5. Burnet PWJ, Eastwood SL, Bristow G, Godlewska BR, Sikka P, Walker M, Harrison PJ (2008): D-amino acid oxidase activity and expression are increased in schizophrenia. Mol Psychiatry 13: 658–660.
6. Verrall L, Walker M, Rawlins N, Bunzel I, Kew JNC, Harrison PJ, Burnet PWJ (2007): D-amino acid oxidase and serine racemase in human brain: Normal distribution and altered expression in schizophrenia. Eur J Neurosci 26:1657–1669.
7. Betts JF, Schweimer JV, Burnham KE, Burnet PWJ, Sharp T, Harrison PJ (2014): D-amino acid oxidase is expressed in the ventral tegmental area and modulates cortical dopamine. Front Synaptic Neurosci 6:11.
8. Saachi S, Binelli G, Pollegioni L (2016): G72 primate-specific gene: A still enigmatic element in psychiatric disorders. Cell Mol Life Sci 73:2029–2039.
9. Howley E, Bestwick M, Fradley R, Harrison H, Leveridge M, Okada K, *et al.* (2017): Assessment of the target engagement and D-serine biomarker profiles of the D-amino acid oxidase inhibitors sodium benzoate and PGM030756. Neurochem Int 42:3779–3288.
10. Piper JD, Piper PW (2017): Benzoate and sorbate salts: A systematic review of the potential hazards of these invaluable preservatives and the expanding spectrum of clinical uses for sodium benzoate. Compr Rev Food Sci Food Safety 16:868–880.