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SHORT COMMUNICATIONS

Effect of 1-aminocyclopentanecarboxylic acid on amino acid utilization in various bacterial test systems

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1-AMINOCYCLOPENTANECARBOXYLIC acid has received considerable attention because of its ability to inhibit the growth of mouse sarcoma-180,[1] carcinoma-755 [1] and Novikoff rat hepatoma.[2] It was submitted to the Cancer Chemotherapy National Service Center by Dr. C. I. Noll of Pennsylvania State University for testing and was assigned the Code Number NSC-1026.[3] Though clinical trials with NSC-1026 were disappointing, the possibility existed of finding new and closely related compounds with improved effectiveness. Recently a number of structures closely related to NSC-1026 have been synthesized and tested against carcinoma-755, sarcoma-180 and leukemia-1210.[4] Almost without exception these derivatives were found to be ineffective. The specificity of the parent compound posed a problem of its mechanism of action.

Because of structural similarity between natural amino acids and NSC-1026, it was tested for the ability to interfere with amino acid utilization of 17 amino acids in a number of bacterial test systems. The amino acids studied were those commonly found on analysis of normal mouse and tumor tissues.[5]

Several bacterial test systems were employed,[6–10] each of which had a specific requirement for the amino acid against which NSC-1026 was tested. Table 1 presents the bacteria used, a reference to the basal medium, the maximum ratio tested of "antagonist" to amino acid, and the effects noted.

The bacterial cultures were provided a complete nutrient medium with sufficient amounts of the amino acid under test to allow approximately one-half maximum growth. A growth antagonist would reduce the growth rate below the half-maximal level if it interfered with the utilization of the particular amino acid in question. If growth exceeded the half-maximal level in the presence of the antagonist

TABLE 1. EFFECT OF 1-AMINOCYCLOPENTANECARBOXYLIC ACID ON THE UTILIZATION OF SEVENTEEN AMINO ACIDS BY BACTERIA

Test organism	Amino acid	Maximum weight ratio of drug to amino acid	Effect on utilization of amino acid	Reference to method and medium used
Lactobacillus delbrueckii LD5	L-phenylalanine	160 : 1	None	(8)
Lactobacillus delbrueckii LD5	L-aspartic	48 : 1	None	(6)
Leuconostoc mesenteroides P-60	L-aspartic	1000 : 1	None	(7)
Streptococcus faecalis R	L-isoleucine	500 : 1	None	(8)
Streptococcus faecalis R	L-valine	500 : 1	None	(8)
Streptococcus faecalis R	L-tyrosine	600 : 1	None	(9)
Streptococcus faecalis R	L-histidine	320 : 1	None	(8)
Streptococcus faecalis R	L-arginine	240 : 1	None	(8)
Streptococcus faecalis R	L-threonine	200 : 1	None	(8)
Streptococcus faecalis R	L-methionine	240 : 1	None	(8)
Streptococcus faecalis R	L-tryptophane	2000 : 1	None	(8)
Lactobacillus arabinosus 17-5	L-leucine	160 : 1	None	(8)
Lactobacillus arabinosus 17-5	L-glutamic	120 : 1	stim. 25%	(10)
Lactobacillus arabinosus 17-5	L-cystine	850 : 1	None	(10)
Lactobacillus arabinosus 17-5	L-serine	200 : 1	None	(7)
Leuconostoc mesenteroides P-60	L-lysine	200 : 1	None	(7)
Leuconostoc mesenteroides P-60	L-proline	200 : 1	None	(7)
Leuconostoc mesenteroides P-60	L-glycine	300 : 1	stim. 20%	(7)

it would be presumed that the antagonist was being metabolized in place of the missing amino acid. Growth was measured by titration of the acid formed in the culture.

An examination of the results in column 4 of Table 1 shows that under the test conditions used, NSC-1026 did not function as an antagonist of any of the 17 amino acids. In the case of *Lactobacillus arabinosus* 17-5 with L-glutamic acid, and *Leuconostoc mesenteroides* P-60 with glycine, there was evidence of some utilization of NSC-1026.

Ross[3] stated "the assumption could be made that NSC-1026 would function as an amino acid antagonist since it can be looked upon as an amino acid lacking the usual α-hydrogen atom". This hypothesis is not substantiated by the data presented in this report.

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The inhibition of gamma aminobutyric-alpha-ketoglutaric acid transaminase *in vitro* and *in vivo* by amino-oxyacetic acid

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THERE is abundant evidence (Roberts and Frankel, 1950; Awapara, *et al.*, 1950; and Roberts, 1956) that γ-aminobutyric acid (GABA) is found in high concentrations in the central nervous system. However, administration of GABA in large doses does not result in increased concentrations of this amino acid in brain, presumably because of its failure to cross the blood-brain barrier (Van Gelder and Elliot, 1958). Baxter and Roberts (1959) demonstrated that inhibition of γ-aminobutyric acid-α-ketoglutaric acid transaminase occurred in rats after administration of suitable doses of hydroxylamine, with concomitant increases of 100 per cent in brain GABA concentrations. This report is concerned with aminoöxyacetic acid (AOAA), a more effective inhibitor of this transaminase.

A transaminase preparation isolated from *E. coli* ATCC-26 was used for testing compounds as potential inhibitors using the following conditions: 40 μ moles of GABA, 40 μ moles of α-ketoglutaric acid (KGA), 50 μ moles of borate buffer, pH 8·2, enzyme sufficient to form from 3 to 4 μ moles of glutamate per hr, and water to 1·5 ml. Analysis for glutamic acid was carried out as described by Baxter and Roberts (1958). Under these conditions, AOAA inhibited the enzyme 100 per cent at a concentration of $3{\cdot}3 \times 10^{-4}$M and 40 per cent at $3{\cdot}3 \times 10^{-6}$M. Further studies with the bacterial preparation revealed that the inhibition was competitive for both substrates of the enzyme. When tested with a transaminase preparation from brain which, in contrast to the bacterial enzyme, requires supplementation with pyridoxal phosphate (Baxter and Roberts, 1958), a 92 per cent inhibition was observed at 1×10^{-5}M.