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STUDY OF THE CELLULAR ACTION OF DRUGS WITH PROTOZOA. I. EFFECT OF 1-AMINO*CYCLO*PENTANE-1-CARBOXYLIC ACID AND 1-AMINO-3-METHYL*CYCLO*HEXANE-1-CARBOXYLIC ACID ON THE PHYTOFLAGELLATE *OCHROMONAS DANICA*

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Abstract—The tumor-inhibiting cyclic amino acids, 1-amino*cyclo*pentane-1-carboxylic acid (ACP) and 1-amino-3-methyl*cyclo*hexane-1-carboxylic acid (AMCH) inhibit the multiplication of *Ochromonas danica*. This inhibition by ACP is annulled by L-alanine and glycine in that order; inhibition by AMCH is annulled by L-leucine.

ON TESTING cyclic amino acids for antitumor properties, one of them, 1-amino*cyclo*pentane-1-carboxylic acid, had antitumor activity in experimental animals;[1, 2] its effect on human tumors is unclear.[3, 4] This compound was marginally effective against various mouse plasmacytomas.[5] Because of its resemblance to amino acids, 1-amino*cyclo*pentane-1-carboxylic acid might inhibit amino-acid metabolism; no direct evidence for its site of action in mammals or tumors has been found. Evidence is presented here for amino-acid metabolism as a site of action of ACP and AMCH on the phytoflagellate *Ochromonas danica*.

EXPERIMENTAL

The organisms used, *Euglena gracilis* strain Z and *O. danica*, were grown in chemically-defined media[6] in 10-ml micro-Fernbach flasks covered with polypropylene tops. The flasks with 5·0 ml of media were placed in rectangular Pyrex glass dishes (19 × 30 cm, internal dimensions), autoclaved, cooled, and inoculated with a drop of a dilute, sterile, distilled water suspension of cells in log growth. The glass dishes were covered with inverted glass dishes and the edges of the dishes sealed with "freezer" tape to exclude dust and permit safe stacking of the sealed trays. Experimental flasks were incubated in light (about 150-foot candles) of cool-white fluorescent lamps at 26° for 5 to 10 days. Multiplication was measured in optical density units (O.D.) with a Welch Densichron equipped with a red-sensitive light probe and a cuvette with a 1-cm light path.

Chemicals were purchased from commercial sources; AMCH and ACP (CB-1639, NSC-1026), the glycine peptide of ACP, and 2-methyl norvaline were obtained through the generosity of Dr. W. C. J. Ross, the Chester Beatty Research Institute. An additional supply of ACP was obtained from the Cancer Chemotherapy National Service Center, Bethesda, Md., U.S.A.

RESULTS

Table 1 shows the effect of the *cyclo*aliphatic compounds and analogs of *cyclo*pentane on the multiplication of the phytoflagellates; only *O. danica* was appreciably sensitive. The natural amino acids were assayed for ability to annul these inhibitions. Inhibition of *O. danica* multiplication by ACP was annulled by glycine and L-alanine only (Table 2); alanine was more effective on a molar basis. DL-N-acetylalanine also

TABLE 1. EFFECT OF SEVERAL DRUGS ON *E. gracilis* AND *O. danica*

Drug	Conc. (mM)	Optical density *E. gracilis*	*O. danica*
AMCH	0·006	2·55	2·30
	0·067	2·44	1·64
	0·64	2·24	0·14
	1·92	2·15	0
	3·8	2·12	0
	6·4	2·09	0
ACP	0·008	2·57	2·69
	0·08	2·55	2·68
	0·8	2·34	1·61
	2·3	1·97	0·11
	4·7	2·04	0
	7·8	1·59	0
Glycine peptide of ACP	0·054	2·54	2·70
	0·54	2·48	2·71
	1·62	2·48	2·17
	3·24	2·48	0·69
	5·44	2·43	0·24
α-Methyl norvaline	0·08	2·52	2·69
	0·8	2·14	2·67
	2·3	1·91	2·29
	4·5	1·61	1·79
	7·5	1·60	1·20

TABLE 2. EFFECT OF AMINO ACIDS ON INHIBITION OF *O. danica* BY 1-AMINO*cyclo*PENTANE-1-CARBOXYLIC ACID (ACP)

	Conc. of amino acids (mM)	Optical density Conc. of ACP (mM) 0·88	1·27	1·66	2·34
0		1·94	0·11	0	0
Glycine	13*	2·32	0·93	0·08	0
L-Alanine	11	2·38	2·24	1·02	0·11
	33	2·32	2·30	1·98	0·16
	66	2·32	2·22	2·25	1·03
D-Alanine	11	1·81	0·10	0	0
	33	2·11	0·16	0	0
	66	0·78	0	0	0

* A higher concentration (39 mM) was inhibitory. Inhibition by glycine (39 mM) was annulled in presence of the above concentrations of DL-alanine.

prevented inhibition by ACP but not so well as DL-alanine; D-alanine and β-alanine were inactive; only L-alanine was effective. Inhibition of *O. danica* multiplication by AMCH was annulled by L-leucine (Table 3); D-leucine was ineffective.

DISCUSSION

ACP inhibits tumors[1–5] in humans and mammals, but its mode of action is unknown. It did not interfere with the utilization of amino acids in several bacteria requiring

TABLE 3. EFFECT OF ISOMERS OF LEUCINE ON INHIBITION OF *O. danica* BY 1-AMINO-3-METHYL*cyclo*HEXANE-1-CARBOXYLIC ACID (AMCH)

	Conc. of amino acids (mM)	Optical density Conc. of AMCH (mM) 0·64	1·92	3·84	6·4
0		2·81	0·74		
L-leucine	0·02	2·86	0·78	0	0
	0·05	2·91	1·62	0	0
	0·08	2·89	2·19	0	0
	0·23	2·85	2·72	1·22	0·14
	0·46	2·83	2·88	2·10	1·66
	0·76	2·87	2·81	2·34	1·38
D-leucine	0·23	2·43	0·34	0	0
	0·46	2·54	0·24	0	0
	0·76	2·26*	0·28	0	0

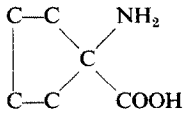
* D-Leucine alone was not significantly inhibitory at this concentration in other trials.

amino acids. In *Leuconostoc mesenteroides* P-60 (requiring glycine) and in *Lactobacillus arabinosus* 17-5 (requiring L-glutamic acid) it enhanced the use of the required amino acids.[7] This suggests that it may have been metabolized by the bacteria or aided transport of the required amino acids. ACP at low concentrations inhibited *Escherichia coli* grown on a salts–glucose medium where all amino acids were endogenously synthesized; this inhibition was annulled by isoleucine, valine, leucine, and threonine, as well as by pantothenate, thiamine, and α-keto-glutaric acid.[8] At high concentrations of this inhibitor only α-keto-β-methylvaleric acid and isoleucine were effective, in that order.[8]

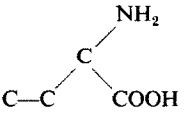
The results with *O. danica* and *E. coli* suggest that ACP interferes with the synthesis or perhaps the transport of several amino acids. The lack of inhibition of bacteria requiring exogenously supplied amino acids[7] may be explained by assuming that this compound interferes with synthesis of amino acids rather than with utilization of preformed amino acids.

AMCH, 1-amino*cyclo*hexane-1-carboxylic acid, or 1-amino-2-methyl*cyclo*hexane-1-carboxylic acid did not inhibit the Walker rat carcinoma; ACP, 1-amino*cyclo*hexane-1-carboxylic acid, and the 1-(N-ethylamino) and 1-(N-methylamino) analogs of *cyclo*hexane-1-carboxylic acid inhibited the growth of Novikoff hepatoma in the rat.[9] AMCH does, however, inhibit multiplication of *O. danica.*

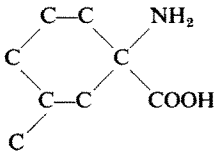
The similarity in structure (Fig. 1) and biological action (inhibition of amino acid metabolism by ACP and AMCH) suggests that these analogs of amino acids block enzymes involved in transport or biosynthesis of these amino acids. Other *cyclo*-aliphatic compounds inhibit metabolism; *e.g.*, *trans*-1,2-*cyclo*pentane dicarboxylic acid inhibited the oxidation of succinate, α-ketoglutarate, and aspartate in *Brucella abortus*[10] and oxidation of succinate in *Tetrahymena pyriformis*.[11]



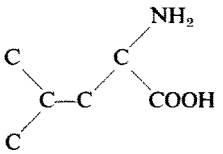
1-Amino*cyclo*pentane-1-carboxylic acid (ACP)



Alanine



1-Amino-3-methyl*cyclo*hexane-1-carboxylic acid (AMCH)



Leucine

FIG. 1. Structure of natural amino acids and their *cyclo*aliphatic analogs compared.

More comparative information on protozoa and mammals is needed, but these results as well as those cited below suggest that protozoa like *O. danica* may offer useful information in screening for cytotoxic agents[12] and for the study of the mode of action at the cellular level of such pharmacologically important compounds as 3-amino-1,2,-4-triazole,[13] hypocholesterolemic compounds,[14] and antimetabolites of acetate.[15]

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