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Regulation of Polyamine Synthesis by Dietary α-Aminoisobutyric Acid and Ornithine (42770)

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Abstract. An experiment was conducted to determine the effect of feeding ornithine in combination with α-aminoisobutyric acid (AIB), an inhibitor of arginase, on the regulation of polyamine synthesis in chicks. A total of 48 chicks with genetically elevated renal arginase activity was fed diets containing crystalline amino acids and 1% AIB with or without 2% ornithine. Feeding AIB reduced renal arginase activity, while renal and hepatic ornithine decarboxylase (ODC) activity increased. Feeding AIB plus ornithine caused no further reduction in renal arginase activity compared with that in chicks fed the AIB-supplemented diet. Renal and hepatic ODC activities, however, fell to below control levels. Renal, hepatic, and breast muscle ornithine concentrations increased substantially when ornithine was fed. AIB plus ornithine increased renal putrescine and spermidine concentrations. It was concluded that AIB could partially overcome the ornithine-induced inhibition of ODC activity. These findings support the hypothesis that dietary manipulation of precursor amino acids of polyamines in the presence of metabolites that induce ODC activity can influence tissue polyamine concentrations. © 1988 Society for Experimental Biology and Medicine.

Polyamines are compounds synthesized from the amino acids arginine and methionine and are required for the growth and development of all cells. It is known that inhibition of polyamine synthesis prevents cell growth and division (1), but the potential for precursor amino acids to regulate polyamine synthesis and thus to control whole animal growth has received little attention. A summary of polyamine metabolism and its regulation has been published by Pegg (1).

Arginase (EC 3.5.3.1., (2)) catalyses the conversion of arginine to ornithine, which is decarboxylated to putrescine in the reaction catalyzed by ornithine decarboxylase (ODC, EC 4.4.4.17.). Arginase activity in the chick is located almost entirely in the kidney although some activity is found in the liver (3). α-Aminoisobutyric acid (AIB) has been shown to inhibit renal arginase activity (4) and to induce ODC activity *in vitro* (5). Recent work by Bedford *et al.* (unpublished) demonstrated that feeding AIB to chicks resulted in such changes *in vivo,* but there was no change in renal putrescine concentration due to a large reduction in tissue concentrations of ornithine, the substrate for ODC. Feeding ornithine has been shown to markedly increase tissue ornithine concentrations, but inhibit ODC activity, resulting in no net accumulation of tissue polyamines (Bedford *et al.,* unpublished). The inhibitory effect of ornithine on ODC activity has previously been shown in cultured cells (5, 6). The objective of the current study was to determine whether tissue ornithine concentrations and ODC activity could be increased simultaneously by feeding ornithine and AIB in combination, in an attempt to increase polyamine synthesis.

Materials and Methods. *Animals and diets.* A total of 48 chicks with genetically elevated renal arginase activity was used in the current study; four chicks per cage, four cages per diet. At 1 week of age, birds were randomly assigned to the dietary treatments. The control diet was based on that of Calvert *et al.* (9) but contained 1.2% arginine, since this has been previously determined to be the requirement of these birds for maximal growth (6). Dietary treatments included the control diet, 1% AIB, and 1% AIB + 2% ornithine, with all amino acid supplements being made at the expense of glutamic acid. Weight gain and food consumption were measured

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for 2 weeks after which time all birds were killed by cervical dislocation, and kidneys, livers, and breast muscle were rapidly excised and immediately frozen in liquid nitrogen. All tissues were stored at −85°C until analyzed.

Enzyme assays. ODC and *S*-adenosylmethionine decarboxylase (AdoMetDC, EC 4.1.1.50) activities were determined by the method of Eloranta *et al.* (10), and arginase activity was assayed according to Smith and Lewis (11), with all methods being modified according to Bedford *et al.* (7).

Analysis of amino acids and polyamines. Renal and hepatic amino acid and polyamine concentrations were determined simultaneously by capillary gas–liquid chromatography with electron capture detection as described previously (7).

Statistical analyses. Data were analyzed according to the general linear models procedure for the analysis of variance (12). A randomized complete block design was used, each block representing a time period of killing in order to remove the effect of diurnal variations in enzyme activity. Orthogonal contrasts were used to separate the means (13).

Results. Food intake was reduced by addition of AIB to the control diet, and further reduced when AIB + ornithine was fed (Table I). Weight gain and food conversion efficiency (FCE) were unaffected by diet.

Renal-soluble protein content was unaffected by diet, but kidney weight as a proportion of body weight was increased by feeding AIB or AIB + ornithine (Table II). Dietary AIB reduced renal arginase activity considerably compared with that of the controls, but no further reduction was noted when AIB + ornithine was fed. Renal ODC activity rose substantially when AIB was included in the diet, but feeding AIB + ornithine resulted in a precipitous decline in ODC activity compared with that of animals fed the AIB-supplemented diet or the control diet. Renal AdoMetDC activity fell when AIB was added to the diet but fell no further when AIB + ornithine was fed (Table II).

Liver weight as a percentage of body weight increased when the AIB-supplemented diet was fed (Table II). Hepatic arginase and AdoMetDC activities were unaffected by dietary treatment. Hepatic ODC activity increased when AIB was fed but decreased below that of the control birds when AIB + ornithine was fed (Table II).

Arginase activity was undetectable in breast muscle, and there were no effects of diet on breast muscle ODC or AdoMetDC activities (Table III).

Renal ornithine concentrations rose more

TABLE I. EFFECT OF DIETARY AIB AND ORNITHINE CONCENTRATION ON CHICK GROWTH, FOOD CONSUMPTION, AND FOOD CONSUMPTION EFFICIENCY

Treatment[a]		Weight gain	Food intake	FCE[b]
AIB (%)	Orn (%)	(g)	(g)	gain/food
0	0	123	229	0.542
1	0	120	217	0.525
1	2	121	183	0.532
SD		5.41	13.72	0.026
Orthogonal contrast[c]				
Control vs AIB		0.3672	0.2472	0.4741
Control vs AIB + Orn		0.8247	0.0012	0.9660
AIB vs AIB + Orn		0.6983	0.0075	0.6430
Parameters tested[d]				
Food intake		0.0026	—	0.6430

Note. Values are means, four birds per replicate, four replicates per diet.
SD = pooled standard deviation.
[a] Dietary concentration of AIB and ornithine (Orn) %.
[b] Food conversion efficiency.
[c] *P* value of contrast of diets tested against one another.
[d] *P* value of other parameters included in the statistical model.

TABLE II. EFFECT OF DIETARY AIB AND ORNITHINE CONCENTRATION ON RENAL AND HEPATIC PROTEIN CONTENT AND ACTIVITIES OF ARGINASE, ORNITHINE DECARBOXYLASE (ODC), AND *S*-ADENOSYLMETHIONINE DECARBOXYLASE (AdoMetDC)

Organ	Treatment[a] AIB (%)	Treatment[a] Orn (%)	Tissue wt/body (%)	Protein content (mg/g)	Arginase[b]	ODC[c]	AdoMetDC[d]
Kidney	0	0	0.91	131	331	2273	996
	1	0	1.05	139	63	5142	831
	1	2	1.04	137	61	1613	751
SD			0.132	13.8	120	2178	185
Orthogonal contrast[e]							
Control vs AIB			0.016	0.205	0.001	0.001	0.039
Control vs AIB + Orn			0.029	0.324	0.001	0.038	0.003
AIB vs AIB + Orn			0.802	0.769	0.974	0.001	0.298
Liver	0	0	2.82	182	58	657	567
	1	0	3.19	186	51	818	582
	1	2	3.07	195	54	219	639
SD			0.31	24.6	9.6	478	161
Orthogonal contrast[e]							
Control vs AIB			0.021	0.688	0.096	0.042	0.829
Control vs AIB + Orn			0.232	0.209	0.431	0.029	0.410
AIB vs AIB + Orn			0.249	0.384	0.363	0.005	0.541

Note. Values are means, $n = 12$ per diet. SD = pooled standard deviation.
[a] Dietary concentration of AIB and ornithine (Orn) %.
[b] μmole urea formed/(min · g protein).
[c] pmole $^{14}CO_2$ formed/(min · g protein).
[d] pmole $^{14}CO_2$ formed/(min · g protein).
[e] *P* value of contrast of diets tested against one another.

than sevenfold in birds fed AIB + ornithine compared with those of birds fed the control or AIB-supplemented diets (Table IV). Renal glutamic acid concentrations fell when the AIB + ornithine diet was fed. Feeding AIB reduced renal lysine concentrations in AIB-

TABLE III. EFFECT OF DIETARY AIB AND ORNITHINE CONCENTRATION ON ACTIVITIES OF ARGINASE, ORNITHINE DECARBOXYLASE (ODC), AND *S*-ADENOSYLMETHIONINE DECARBOXYLASE (AdoMetDC) IN BREAST MUSCLE

Treatment[a] AIB (%)	Treatment[a] Orn (%)	Arginase[b]	ODC[c]	AdoMetDC[d]
0	0	ND[e]	341	473
1	0	ND	483	525
1	2	ND	346	540
SD		ND	224	322
Orthogonal contrast[f]				
Control vs AIB			0.135	0.697
Control vs AIB + Orn			0.953	0.615
AIB vs AIB + Orn			0.149	0.909

Note. Values are means, $n = 12$ per diet. SD = pooled standard deviation.
[a] Dietary concentration of AIB and ornithine (Orn) %.
[b] μmole urea formed/(min · g protein).
[c] pmole $^{14}CO_2$ formed/(min · g protein).
[d] pmole $^{14}CO_2$ formed/(min · g protein).
[e] Not detectable.
[f] *P* value of contrast of diets tested against one another.

TABLE IV. EFFECT OF DIETARY AIB AND ORNITHINE CONCENTRATION ON RENAL AND HEPATIC CONCENTRATIONS OF POLYAMINES AND AMINO ACIDS (μmole/g TISSUE)

	Tissue							
	Kidney				Liver			
Dietary treatment[a]								
AIB	0	1	1	SD	0	1	1	SD
Ornithine	0	0	2		0	0	2	
Metabolite								
Arginine	0.365[b]	0.510	0.620	.307	0.401	0.398	0.339	.299
Ornithine	0.761	0.522	4.079	.722	0.320	0.324	1.878	.289
Glutamate	31.43	27.23	23.34	8.38	18.37	16.34	15.79	3.25
Proline	1.122	1.315	1.382	.473	1.163	1.005	1.297	.532
Methionine	0.885	0.905	0.935	.495	0.885	0.898	1.028	.509
Lysine	2.548	1.900	3.435	1.10	2.026	1.430	1.527	.648
Putrescine	0.236	0.250	0.439	.205	0.475	0.525	0.675	.256
Spermidine	0.159	0.137	0.282	.126	0.380	0.281	0.336	.183
Spermine	0.150	0.155	0.248	.199	0.266	0.206	0.175	.195

Statistical summary

	Orthogonal contrast[c]					
	Kidney			Liver		
Dependent variables	1 vs 2	1 vs 3	2 vs 3	1 vs 2	1 vs 3	2 vs 3
Arginine	0.300	0.074	0.429	0.987	0.683	0.694
Ornithine	0.466	0.001	0.001	0.979	0.001	0.001
Glutamate	0.292	0.048	0.328	0.238	0.127	0.713
Proline	0.372	0.231	0.753	0.560	0.618	0.285
Methionine	0.929	0.824	0.894	0.959	0.579	0.614
Lysine	0.198	0.082	0.005	0.082	0.139	0.768
Putrescine	0.881	0.036	0.049	0.702	0.134	0.256
Spermidine	0.649	0.039	0.016	0.292	0.632	0.559
Spermine	0.932	0.072	0.086	0.495	0.300	0.717

Note. SD = pooled standard deviation.
[a] Dietary concentration of AIB and ornithine (Orn) %.
[b] Values are means, $n = 10$.
[c] P value of contrast of diets tested against one another (1 = Control diet, 2 = 1% AIB diet, 3 = 1% AIB and 2% ornithine diet).

fed birds compared with those of birds fed AIB + ornithine. The combination of AIB + ornithine led to increased renal putrescine and spermidine concentrations compared with those of the other two treatments. Spermine concentrations were unaffected by diet.

Hepatic ornithine concentrations were increased when AIB + ornithine was fed (Table IV). Dietary treatment did not affect the hepatic concentrations of arginine, glutamic acid, proline, methionine, or any of the polyamines.

Amino acid and polyamine concentrations were also measured in breast muscle. Ornithine concentrations rose significantly when AIB + ornithine was fed compared with those of the other treatments. Putrescine concentrations, although found to be unchanged by diet, were very high (1.5 to 2 μmole/g) compared to concentrations in kidney or liver.

Discussion. *Enzyme activities.* Reductions in renal arginase activity have been observed when either ornithine (Bedford *et al.*, unpublished) or AIB (4) was fed. In the present experiment, however, feeding AIB + ornithine resulted in no further reduction in renal arginase activity when compared with

that of birds fed AIB only. This lack of additivity suggests that AIB and ornithine may inhibit renal arginase through the same biochemical mechanism.

The substantial activation of renal ODC by dietary AIB confirms previous work in the chick (Bedford *et al.*, unpublished) and in cell culture (5). The feeding of 2% ornithine, in contrast, has been shown to reduce renal ODC activity by 70% (Bedford *et al.*, unpublished). When AIB + 2% ornithine was fed in the current experiment, ODC activity was reduced by only 29%. This could result in greater synthesis of putrescine compared with that of chicks fed ornithine alone.

Hepatic arginase activity was unaffected by dietary treatment which confirms previous observations when either AIB or ornithine was fed (Bedford *et al.*, unpublished). The combination of AIB + ornithine reduced hepatic ODC activity far below that of controls, while the reduction was only moderate in the kidney. Hepatic AdoMetDC activity was not affected by dietary manipulation, but there was a decrease in renal AdoMetDC activity with inclusion of AIB and a further decrease when AIB + ornithine was fed. This illustrates that there are tissue differences in the regulation of polyamine synthesis.

Breast muscle enzyme activities were unaffected by dietary treatment, although there was a trend toward increased ODC activity with the feeding of AIB. The lack of response to diet was expected as this tissue has previously been shown to be unresponsive to dietary treatments that have had significant effects on polyamine synthetic enzymes in kidney and liver (7).

Metabolite concentrations. In a previous study in which ornithine was fed in the absence of AIB, renal concentrations of ornithine increased with increments of dietary ornithine (Bedford *et al.*, unpublished). This did not result in increased renal putrescine concentrations, however, since renal ODC activity was reduced substantially. Ornithine has previously been implicated as a metabolite that may inhibit the activity of ODC (7, 8). The feeding of AIB + ornithine partially overcame this reduction in renal ODC activity and thus resulted in a significant increase in renal putrescine concentration which in turn increased spermidine concentrations. The cellular concentration of spermidine may regulate spermine synthesis to some degree since spermine concentrations tended to be elevated ($P = 0.072$) in birds fed the AIB + ornithine diet despite the observed inhibition of AdoMetDC activity.

Although hepatic ornithine concentrations increased with feeding of AIB + ornithine, the magnitude of increase in tissue ornithine concentrations was much greater in the kidney compared with those of the liver. In addition, since ODC activity was depressed to a greater extent in the liver compared with that of the kidney when this diet was fed, the increase in hepatic putrescine concentration was not statistically significant.

Since there were no significant differences in hepatic putrescine concentrations, and hepatic AdoMetDC activities were not influenced by diet, it is not surprising that hepatic spermidine and spermine concentrations were also unaffected by diet.

Ornithine concentrations in breast muscle rose as expected with inclusion of ornithine in the diet, but, as in the liver, this did not result in an accumulation of putrescine despite the fact that ODC and AdoMetDC activities were not influenced by diet. It is interesting to note the extremely high putrescine concentrations in breast muscle compared with those of the liver and kidney. Since the activities of the polyamine synthetic enzymes are so low in breast muscle compared with those in the liver and kidney, it is possible that some of the putrescine in breast muscle has been transported from other tissues such as kidney and liver.

It can be concluded that feeding AIB and ornithine in combination raised renal ornithine concentrations and maintained ODC activity thereby increasing putrescine and spermidine concentrations in the kidney. This supports the hypothesis that polyamine synthesis can be regulated, in part, through substrate induction.

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