Non-metabolizable amino acids are potent stimulators of hepatic and renal ornithine decarboxylase activity

Elliott W. CHIDECKEL and Diane EDWARDS

Department of Medicine, West Virginia University Medical School, Morgantown, WV 26506, U.S.A.

(Received 19 October 1982/Accepted 21 December 1982)

The non-metabolizable amino acids α -aminoisobutyric acid (AIB) and cycloleucine and the poorly metabolizable amino acid D-alanine potently stimulated hepatic ornithine decarboxylase (ODC) activity in starved rats. The stimulation by AIB was shown to have several of the characteristics of stimulation by a protein meal and occurred in hypophysectomized animals. AIB also stimulated renal, but not brain or heart, ODC activity.

Re-feeding protein to a starved animal results in a large increase in hepatic ODC activity (Fausto, 1971). This increase occurs in hypophysectomized (Fausto, 1971) and diabetic rats (Rosovski *et al.*, 1979) and appears to be a direct effect of ingested amino acids.

AIB is a non-metabolized analogue of alanine (Christensen *et al.*, 1956). It is an inhibitor of amino acid transport across the cell membrane, and when administered to the rat causes depletion of other neutral amino acids in the liver (Christensen & Cullen, 1968) and kidney (McInnes & Sriver, 1979). In order to understand the mechanism through which amino acids affect polyamine metabolism, AIB was administered to starved rats. The actions of another non-metabolizable amino acid, cycloleucine (Sterling *et al.*, 1962), and of the poorly metabolizable amino acid, D-alanine (Birnbaum *et al.*, 1957), were also investigated.

Materials and methods

The studies were performed on male Sprague–Dawley rats. All rats weighed between 180 and 220 g at the time of death. Food was removed in the afternoon and the rats were killed by decapitation on the morning of day 3 after food removal. Hypophysectomized rats were obtained from Zivic Miller (Attisison Park, PA, U.S.A.). When these latter rats were starved, the same schedule as for the intact rats was utilized except that these rats received dexamethasone, 0.01 mg/kg per day subcutaneously, during the 3 days of starvation, to ensure their survival.

AIB, L-alanine, cycloleucine and D-alanine were all obtained from Sigma Chemical Co., St. Louis,

Abbreviations used: ODC, ornithine decarboxylase (EC 4.1.1.17); AIB, α -aminoisobutyrate.

MO, U.S.A., and 250 mg of each substance was dissolved in 10 ml of water and injected intraperitoneally into the rats. Control rats received 10 ml of water or 0.9% NaCl. The right lobe of the liver, the outer third of the kidney, including both cortex and medulla, the neocortex of the brain and the apical third of the heart were sampled for ODC activity; $n = 5$ –6 for each treatment and at each time point. Cycloheximide was obtained from Calbiochem, La Jolla, CA, U.S.A., and actinomycin D was from Merck, Sharp and Dohme, West Point, PA, U.S.A.

ODC was assayed by the method of Russell & Snyder (1968), with modification (Chideckel *et al.*, 1980). A half-saturating concentration of unlabelled ornithine was used in the assay to minimize the effect of endogenous changes of ornithine in the tissues. Glucose was measured enzymically in a Beckman glucose analyser and insulin was assayed by a standard radioimmunoassay by using rat insulin standard and ethylene glycol separation.

Results and discussion

After administration of AIB to 3-day-starved rats, hepatic ODC activity was markedly stimulated, reaching a peak at 100-fold basal values at 4 h after administration and subsiding by 6–8 h (Fig. 1). The utilizable amino acid L-alanine given in the same dose was also stimulatory. Other non-metabolizable or poorly metabolizable but transportable amino acids were examined. Cycloleucine and D-alanine both had potent effects on ODC activation in the 3-day-starved rat (Fig. 1).

Hepatic ODC activity responds positively to somatotropin (growth hormone), glucocorticoids, insulin and glucagon (Panko & Kenney, 1971). In order to assess the directness of AIB's effect,

Fig. 1. Effect of administration of non-metabolizable amino acids and L-alanine on hepatic ODC activity in the 3-day-starved rat; effect of AIB administration in the hypophysectomized (hypox) 3-day-starved rat

Each time point represents the mean $\pm$ S.E.M. for five or six rats. By one-way analysis of variance and the Newman-Keuls manoeuvre (Snedecor & Cochran, 1976), AIB, cycloleucine, D-alanine, and L-alanine caused at 4 h a significant ($P < 0.05$) increase in ODC activity over the starved control, as well as over the water-treated and saline-treated controls. The AIB-induced increase in ODC activity at 4 h was greater than that ($P < 0.05$) of the other amino acids. By similar statistics ODC activity at 2, 4 and 6 h after AIB administration was significantly ($P < 0.05$) increased over the zero-time starved control. By Student's *t* test ODC activity at 4 h was significantly greater ($P < 0.001$) in the AIB-treated hypophysectomized rats than in the hypophysectomized control (not shown; ODC activity was barely detectable). Thus non-metabolizable amino acids as well as L-alanine are potent stimulators of hepatic ODC activity in starved and hypophysectomized-starved rats.

hypophysectomized rats, placed on daily glucocorticoid replacement, were starved for 3 days. The lack of mediation by somatotropin or glucocorticoids was proved by the ability of AIB to stimulate hepatic ODC activity markedly in this model (Fig. 1).

It is very unlikely that AIB's effect on ODC activity was mediated by insulin. Though AIB can stimulate insulin release, it does not cause the degree of hyperinsulinaemia required to stimulate ODC activity. In fact, in the 3-day-starved rats 4 h after receiving AIB, serum glucose (mmol/l) was 7.1 ± 0.1 (mean $\pm$ S.E.M.) compared with 6.8 ± 0.2 in control 3-day-starved animals, and serum insulin (μ units/ml) was 22.3 ± 1 compared with 17.2 ± 0.5 in controls. This slight but significant ($P < 0.05$ by Student's *t* test) increase in insulin is well below that achieved by a glucose meal in 3-day-starved animals, which has

Table 1. Effect of AIB or saline on ODC activity in liver, kidney or heart under various conditions
For details see the text.

Tissue	Condition	ODC activity (pmol of CO ₂ /h per mg of protein) (mean $\pm$ S.E.M.) 4 h after administration	
		AIB	Saline
Liver†	Starved	1088 $\pm$ 124	7 $\pm$ 1
	Starved + actinomycin D, 4 h*	145 $\pm$ 53	
	Starved + cycloheximide, 4 h*	1 $\pm$ 0	
	Starved + cycloheximide, 30 min*	687 $\pm$ 102	
Kidney‡	Starved	1259 $\pm$ 35	87 $\pm$ 16
Brain§	Starved	5 $\pm$ 2	4 $\pm$ 1
Heart§	Starved	63 $\pm$ 10	77 $\pm$ 14

* Time elapsed between drug administration and killing the rat.

† By one-way analysis of variance and the Newman-Keuls manoeuvre (Snedecor & Cochran, 1976), all treatment groups were significantly ($P < 0.05$) different from the starved/AIB group, which was significantly different from the 'saline' group.

‡ AIB versus saline, by Student's *t* test, $P < 0.001$.

§ AIB versus saline, non-significant.

no effect on hepatic ODC activity (Chideckel, 1982). Glucagon is an unlikely mediator; it does not stimulate hepatic ODC activity to the extent seen with AIB (Panko & Kenney, 1971). Also in favour of AIB's effect being direct is the observation (Viceps-Madore *et al.*, 1982) that AIB stimulates ODC activity in cells of a neuroblastoma tissue culture.

AIB shares several characteristics with a protein meal and other stimuli (Russell, 1981) of hepatic ODC activity. (a) In our laboratory, in the 3-day-starved rat, a protein meal causes hepatic ODC activity to reach a peak at 4 h and approach baseline by 8 h (Chideckel, 1982). Stimulation by AIB follows the same time course (Fig. 1). (b) Protein-meal stimulation of hepatic ODC can be blocked by cycloheximide and actinomycin D (Noguchi *et al.*, 1979). ODC activation by AIB was blocked by cycloheximide (50 mg/kg, intraperitoneally) or actinomycin D (2 mg/kg, intraperitoneally), when either was given simultaneously with the AIB (Table 1). (c) It has been demonstrated that amino acids added to hepatoma-cell tissue (Hogan & Murden, 1974) or to an isolated perfused liver (Jefferson & Pegg, 1977) stimulate ODC activity, and the stimulation is in part due to prolongation of ODC's half-life. In the 3-day-starved rat, AIB was ad-

ministered; 30 min before the rat was killed at 4 h, cycloheximide (50 mg/kg) was also administered by intraperitoneal injection. Cycloheximide caused only a 37% decline in hepatic ODC activity (Table 1), consistent with a half-life greater than that of 10–20 min noted by Russell & Snyder (1969) under basal or somatotropin-stimulated conditions.

The specificity of AIB's effect on ODC activity is demonstrated by its inability to stimulate brain or heart ODC (Table 1). Renal ODC activity is markedly stimulated (Table 1). The liver and kidney are the two organs that accumulate AIB to the greatest degree (Christensen & Cullen, 1968; McInnes & Scriver, 1979).

These studies demonstrate that in the whole animal amino acid metabolism may not be required for amino acid stimulation of hepatic and renal ODC activity. They suggest that the mechanism of the protein-meal stimulation of hepatic ODC activity may be similar to the stimulation by the non-metabolizable amino acid AIB. When first inspected, such a similarity may appear contradictory: a protein meal floods the hepatocytes with metabolizable amino acids, whereas AIB causes hepatocyte depletion. However, both stimuli cause the hepatocyte to remain in contact for a matter of hours with a substantial quantity of neutral amino acid. Indeed, hepatic AIB has been shown to reach 44 mmol/kg after administration of a dose only slightly greater than that used in the present experiments (Christensen & Cullen, 1968). It may be that such prolonged contact is the stimulus for ODC activation.

This work was supported by the West Virginia Medical Corporation and the N.I.H. (grant AM60620). We are

grateful for the excellent technical help of Doris Brown-Hogan and Susie Rogers.

References

- Birnbaum, S. M., Winitz, M. & Greenstein, J. B. (1957) *Arch. Biochem. Biophys.* **72**, 428–436
- Chideckel, E. W. (1982) *Metab. Clin. Exp.* in the press
- Chideckel, E. W., Rozovski, S. J. & Belur, E. R. (1980) *Biochem. J.* **192**, 765–767
- Christensen, H. N. & Cullen, A. M. (1968) *Biochim. Biophys. Acta* **150**, 237–252
- Christensen, H. N., Aspen, A. J. & Rice, E. G. (1956) *J. Biol. Chem.* **220**, 287–294
- Fausto, N. (1971) *Biochim. Biophys. Acta* **238**, 116–128
- Hogan, B. L. M. & Murden, S. (1974) *J. Cell. Physiol.* **83**, 345–352
- Jefferson, L. S. & Pegg, A. E. (1977) *Biochim. Biophys. Acta* **484**, 177–187
- McInnes, R. R. & Scriver, C. R. (1979) *Am. J. Physiol.* **237**, F274–F284
- Noguchi, T., Aramaki, Y., Kameji, T. & Hayashi, S. (1979) *J. Biochem. (Tokyo)* **85**, 953–959
- Panko, W. B. & Kenney, F. T. (1971) *Biochem. Biophys. Res. Commun.* **43**, 346–350
- Rosovski, S. J., Conover, C. A. & Ruderman, N. B. (1979) *Life Sci.* **25**, 553–560
- Russell, D. H. (1981) *Med. Biol.* **59**, 286–295
- Russell, D. H. & Snyder, S. H. (1968) *Mol. Pharmacol.* **5**, 253–262
- Russell, D. H. & Snyder, S. H. (1969) *Proc. Natl. Acad. Sci. U.S.A.* **60**, 1420–1427
- Snedecor, G. W. & Cochran, W. G. (1976) *Statistical Methods*, pp. 258–275, Iowa State University Press, Ames, IA
- Sterling, W. R., Henderson, J. F., Mandel, H. G. & Smith, P. K. (1962) *Biochem. Pharmacol.* **11**, 135–145
- Viceps-Madore, D., Chen, K. Y., Tsau, H. & Canellakis, E. S. (1982) *Biochim. Biophys. Acta* **717**, 305–315