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DECARBOXYLATION OF ORNITHINE AND LYSINE IN RAT TISSUES

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Summary

The possibility that arginine and lysine might be decarboxylated by rat tissues was investigated. No evidence for decarboxylation of arginine could be found. Lysine decarboxylase (L-lysine carboxy-lyase, EC 4.1.1.18) activity producing CO_2 and cadaverine was detected in extracts from rat ventral prostate, androgen-stimulated mouse kidney, regenerating rat liver and livers from rats pretreated with thioacetamide. These tissues all have high ornithine decarboxylase (L-ornithine carboxy-lyase, EC 4.1.1.17) activities. Lysine and ornithine decarboxylase activities were lost to similar extents on inhibition of protein synthesis by cycloheximide and on exposure to α-difluoromethylornithine. A highly purified ornithine decarboxylase preparation was able to decarboxylate lysine and the ratio of ornithine to lysine decarboxylase activities was constant throughout purification. Kinetic studies of the purified preparation showed that the V for ornithine was about 4-fold greater than for lysine, but the K_m for lysine (9 mM) was 100-times greater than that for ornithine (0.09 mM). These experiments indicate that all of the detectable lysine decarboxylase activity in rat and mouse tissues was due to the action of ornithine decarboxylase and that significant cadaverine production in vivo would occur only when ornithine decarboxylase activity is high and lysine concentrations substantially exceed those of ornithine.

Introduction

Mammalian cells produce putrescine (1,4-diaminobutane) by the action of an ornithine decarboxylase (L-ornithine carboxy-lyase, EC 4.1.1.17) [1,2]. Although there have been very many studies of changes in the activity of ornithine decarboxylase in response to a wide variety of stimuli (reviewed by

Refs. 3—6) and substantial purifications of this enzyme have now been achieved from rat prostate [7], rat liver [8,11] and mouse fibroblasts [12], there has been surprisingly little consideration of the substrate specificity of this enzyme or of the possibility that other decarboxylases forming diamines might be present in mammalian cells. It is well known that microorganisms and plants can decarboxylate both lysine forming cadaverine and arginine forming agmatine [3,4,13]. Pegg and Williams-Ashman [1] reported that crude extracts from rat ventral prostate, which contained relatively high ornithine decarboxylase activities were unable to decarboxylate L-arginine and two laboratories claimed that decarboxylation of L-ornithine by rat liver extract was not influenced by the addition of 10 mM lysine [2,8]. More recently, evidence that cadaverine (1,5-diaminopentane) may be produced in chick embryos and mouse tissues, particularly the androgen-stimulated kidney has been published [14—18] and it was suggested that cadaverine production in such mice is mediated via the decarboxylation of lysine by ornithine decarboxylase [15].

The possible production of decarboxylated derivatives of arginine and lysine in mammalian cells is of particular importance since many papers have suggested that the polyamines, spermidine and spermine appear to play an important role in cell growth [3—6,19]. Putrescine is known to be produced from agmatine in microorganisms and plants [3—5] and cadaverine appears to be able to substitute partially for putrescine in formation of a spermidine analog under conditions of putrescine deprivation in microorganisms [4,5,20]. In the present paper, the decarboxylation of these amino acids by mammalian cell extracts has been investigated and the specificity of a highly purified rat liver ornithine decarboxylase preparation towards amino acids and inhibition by diamines has been examined.

Experimental procedures

Materials. L-[1-^{14}C]Ornithine (46 Ci/mol), DL-[1-^{14}C]ornithine (45 Ci/mol), L-[U-^{14}C]lysine (301 Ci/mol), L-[U-^{14}C]arginine (305 Ci/mol) and [1,5-^{14}C]-cadaverine (98 Ci/mol) were purchased from New England Nuclear. DL-[1-^{14}C]-Lysine (24 Ci/mol) was purchased from the Radiochemical Centre, Amersham, U.K. α-Difluoromethylornithine was a generous gift from Centre de Recherche Merrell International, Strasbourg, France. All other biochemicals were obtained from Sigma. Female Sprague-Dawley rats (150—300 g) were obtained from Charles River Laboratories. Normal mice of the strain originated by Dr. S. Ohno were obtained from Dr. P. Feil (Department of Endocrinology).

Purity of radiochemicals. The labeled lysine preparations were analyzed for purity by running 1 μCi aliquots on a Beckman amino acid analyzer. More than 98% of the radioactivity was recovered in the lysine peak. There was no radioactivity (less than 10 cpm above background) in the fractions corresponding to ornithine. The [U-^{14}C]lysine was contaminated with a small amount of radioactive material which migrated as cadaverine in high voltage paper electrophoresis, paper chromatography and on the amino acid analyzer. This radioactivity, although only 0.1% of the total, provided an inconveniently high blank for studies of cadaverine production and was removed by running all of the [U-^{14}C]-lysine on paper chromatography (see below) and eluting the region

corresponding to lysine with 0.01 N HCl. The eluate was concentrated by evaporation under reduced pressure and used for the assays.

Assays of decarboxylase activity. Tissue extracts were prepared by homogenization in 4 vols. ice-cold 25 mM Tris-HCl, 2.5 mM dithiothreitol, 0.1 mM disodium EDTA (pH 7.5). After centrifugation at 2°C for 30 min at 30 000 × *g*, the supernatant was used as enzyme source. In some experiments the extracts were dialyzed overnight against the homogenizing buffer before use. Activity of ornithine decarboxylase was measured by incubation of a total assay volume of 0.25 ml containing 0.4 mM L-ornithine, 0.04 mM DL-[1-^{14}C]ornithine (45 Ci/mol), 0.04 mM pyridoxal phosphate, 2.5 mM dithiothreitol, 0.06 mM EDTA and 50 mM Tris-HCl (pH 7.5). After incubation at 37°C, the reaction was halted by addition of 0.1 ml 20% (w/v) trichloroacetic acid and the $^{14}CO_2$ released was trapped in 0.25 ml 1 N hyamine hydroxide suspended above the reaction mixture in a polypropylene well (Kontes). A further 30 min incubation at 37°C in a shaking water bath was used to ensure complete absorption of the $^{14}CO_2$ and the well was transferred to a scintillation vial containing 10 ml toluene-based scintillation fluid and the radioactivity present was counted at an efficiency of 45%. When kinetic measurments on highly purified ornithine decarboxylase were made, the DL-[1-^{14}C]ornithine was replaced by L-[1-^{14}C]-ornithine. The assays also contained 1 mg/ml bovine serum albumin which was found to stabilize the activity and the enzyme was preincubated for 5 min with the pyridoxal phosphate before addition of the amino acid. When lysine decarboxylase (L-lysine carboxy-lyase, EC 4.1.1.18) activity of the purified ornithine decarboxylase was assayed, the ornithine was replaced by L-[U-^{14}C]lysine. Lysine decarboxylase activities of crude tissue extracts was measured using a total assay volume of 1 ml to allow for a greater amount of tissue extract to be added and the L-lysine concentration was 0.5 mM with 2 μCi DL-[1-^{14}C]lysine or L-[U-^{14}C]lysine/ml. 0.05 mM *N,N'*-diaminoguanidine was added to some assays to prevent any diamine oxidase activity from degrading any diamine formed.

Production of [^{14}C]cadaverine was determined by transferring the trichloroacetic acid-precipitated extracts to glass centrifuge tubes using 5 ml water as a wash to ensure quantitative transfer. 50 nmol unlabeled cadaverine was then added as carrier, the denatured protein removed by centrifugation and the supernatant applied to a small column (0.4 × 5 cm) of Dowex-50-H$^+$. The column was washed with 3 ml water, 10 ml 0.5 N HCl and 5 ml 4 N HCl. The 4 N HCl eluate was evaporated to dryness, dissolved in 0.1 ml 0.01 N HCl and the cadaverine separated by paper electrophoresis or chromatography. Paper electrophoresis was run at 3500 V for 1 h using Whatman 3 MM paper and 0.065 M sodium sulfosalicylate buffer (pH 3.2) in a water cooled flat-plate apparatus. Descending paper chromatography was run in $C_2H_5OH/NH_4OH/H_2O$ (18 : 1 : 1, v/v). R_F values were α-ketoadipate, 0.02; α-aminoadipate, 0.10, lysine, 0.20; cadaverine, 0.55. Markers were detected by staining with ninhydrin. After drying the papers were cut into strips 1 cm wide and the radioactivity present determined by scintillation counting in a toluene-based scintillation fluid. Efficiency of counting of ^{14}C on the paper was around 40%.

Treatment of animals. Liver extracts were prepared from rats subjected to two-thirds partial hepatectomy 4 h before death and from rats given 150 mg/kg

doses of thioacetamide by intraperitoneal injection of a solution containing 50 mg/ml in 0.9% (w/v) NaCl 20—24 h before death. Adult mice (20—30 g) were treated with excess androgens by subcutaneous injection of 2 mg testosterone enanthrate 3 times a week for 2 weeks.

Purification of ornithine decarboxylase. Ornithine decarboxylase was purified from liver of rats pretreated with thioacetamide by a method based on that of Hölttä [9] but incorporating an affinity chromatography step. The extract was fractionated with $(NH_4)_2SO_4$ and on DEAE-cellulose [9]. The enzyme was then precipitated by addition of $(NH_4)_2SO_4$ to 50% saturation, dissolved in 25 mM Tris-HCl (pH 7.5), 2.5 mM dithiothreitol, 0.05 M NaCl, dialyzed for 6 h, and applied to a column (1.5 X 12 cm) of Sepharose linked to (5′-phosphopyridoxyl) ornithine. After elution with the loading buffer and the same buffer containing 0.15 M NaCl, only 10% of the applied ornithine decarboxylase was not bound. The column was then eluted with the loading buffer containing 1 mM L-ornithine and 0.5 mM pyridoxal phosphate, which displaced the ornithine decarboxylase. Approx. 60% of the applied activity was recovered in the eluate from the affinity column and 10% was not bound. The remaining 30% was not accounted for and may be inactivated during loading (since ornithine decarboxylase is quite unstable in the absence of pyridoxal phosphate [1,10]) or retained on the column in some form which cannot be eluted. The enzyme was then further purified by chromatography on Sephadex G-150 as previously described [9] with the exception that 0.05 mM pyridoxal phosphate was included in the buffer. The final enzyme preparation had a specific activity of 13.5 μmol $^{14}CO_2$ produced in 1 h by 1 mg protein under the standard assay conditions. This represents a 7800-fold purification in 19% yield (Table IV).

The affinity absorbent was prepared by coupling pyridoxal phosphate to Sepharose via the 3-O-position as described by Ikeda et al. [21]. The ornithine adduct of this material was then prepared essentially as described for the tryptophan complex [22]. The Sepharose-pyridoxal 5′-phosphate was suspended in 0.1 M sodium phosphate buffer (pH 8.5), 25 mM L-ornithine. 100 mg $NaBH_4$ was then added to reduce the Schiff base. After 2 h, the gel was washed extensively with 0.1 M phosphate buffer (pH 5 and 8.5, alternately) and stored in the presence of 1 M NaCl in 25 mM Tris-HCl (pH 7.5). The bound pyridoxal phosphate content of the gels was calculated from the spectral characteristics as approx. 0.9 μmol/ml gel [21]. Since we did not use an N-δ-blocked ornithine derivative in this preparation, it is probable that a substantial part of the *N*-(5′-phosphopyridoxyl)ornithine is attached via the δ- rather than the α-amino group. Because of the difficulty in removing an N-δ-blocking group without degrading the Sepharose adduct and because the adduct prepared as described above was effective in the purification we did not attempt to prepare the specific α-linked product.

General methods. Protein was measured by the Lowry method [23] with crystalline bovine serum albumin as standard. Where necessary, protein was precipitated with trichloroacetic acid to prevent interference by dithiothreitol [7]. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate was carried out as described by Weber and Osborn [24].

Results

When crude, dialyzed supernatant extracts from homogenates of various rat tissues were incubated with 0.5 mM [1-^{14}C]lysine in the presence of 0.04 mM pyridoxal phosphate, $^{14}CO_2$ was produced (Table I). The activity was very low and liver, kidney and ventral prostate (Table I) had at least four-times the activity of other tissues including brain, spleen, intestine and heart. Activity in the liver extracts was enhanced almost 100% when measured in extracts from rats pretreated with thioacetamide or previously subjected to partial hepatectomy. These treatments greatly increase L-ornithine decarboxylase activity in such extracts (Refs. 6, 8, and Table I). There was, therefore, no correlation between $^{14}CO_2$ production from carboxyl-labeled ornithine and carboxyl-labeled lysine in these extracts since prostate has a much higher ornithine decarboxylase activity than control liver or kidney whereas $^{14}CO_2$ production from [1-^{14}C]lysine was similar. Also, treatments increasing ornithine decarboxylase activity 75-fold in liver extracts increased the putative lysine decarboxylation only 2-fold at most. However, when [U-^{14}C]lysine was used as a substrate and [^{14}C]cadaverine production was measured, it was found that virtually all of the $^{14}CO_2$ production by kidney and unstimulated liver extracts was not associated with [^{14}C]cadaverine production. Cadaverine formation by these tissues was below the limit of detection. Definite cadaverine formation was observed with the prostate extracts and the liver extracts stimulated by partial hepatectomy or thioacetamide (Table I). The identity of this material as cadaverine was confirmed by its co-migration with authentic compound on paper chromatography, paper electrophoresis and separation by an amino acid analyzer. The activity cannot be due to decarboxylation of a labeled ornithine contaminant of the [U-^{14}C]lysine since cadaverine and putrescine are easily separeated by these procedures and no labeled ornithine was found when the lysine preparation was tested on the amino acid analyzer. The possibility that the control liver and kidney preparations did produce amounts of [^{14}C]-

TABLE I

DECARBOXYLATION OF ORNITHINE AND LYSINE AND FORMATION OF CADAVERINE FROM LYSINE BY RAT TISSUE EXTRACTS

Extracts were prepared from control rat liver, rat liver from rats subjected to partial hepatectomy 4 h previously, rat liver from rats injected with 150 mg/kg thioacetamide 24 h previously, and from kidneys and ventral prostates of control rats. Formation of $^{14}CO_2$ from [1-^{14}C]ornithine or [1-^{14}C]lysine (decarboxylase activity) and formation of [^{14}C]cadaverine from [U-^{14}C]lysine were measured. N.D., not detected.

Source of extract	Ornithine decarboxylase activity (nmol $^{14}CO_2$/ mg protein per h)	Lysine decarboxylase activity (pmol $^{14}CO_2$/ mg protein per h)	Cadaverine formation (pmol/mg protein per h)
Liver	0.02	18	N.D.
4 h regenerating liver	1.53	35	17
Thioacetamide-stimulated liver	1.47	36	16
Ventral prostate	1.72	20	19
Kidney	0.19	24	N.D.

cadaverine stoichiometric with the $^{14}CO_2$ released, but that the cadaverine was further degraded was eliminated by the finding that when comparable amounts of cadaverine were incubated with the extracts under the same conditions, more than 90% was recovered. These results, therefore, suggest that most of the production of $^{14}CO_2$ from [1-^{14}C]lysine by liver and kidney is due to other metabolic reactions and not to a lysine decarboxylase forming cadaverine, but that lysine decarboxylase activity does exist in rat prostate and in regenerating or thioacetamide-treated rat liver.

Evidence supporting this hypothesis was obtained by studying the effects of various inhibitors on the production of $^{14}CO_2$ from [1-^{14}C]lysine by various tissue extracts. As shown in Table II, the addition of 1 mM α-aminoadipate or α-ketoadipate, which are known metabolites of L-lysine on the metabolic degradative pathway leading to loss of the α-carbon as CO_2 [16,25,26], inhibited $^{14}CO_2$ production from [1-^{14}C]lysine by more than 90% in control liver extracts, but by only 50—60% in regenerating liver extracts and only 10% in prostate extracts. Cadaverine production from lysine by regenerating liver and prostate extracts was not significantly inhibited by α-ketoadipate. However, cadaverine production was prevented by 0.1 mM α-difluoromethylornithine. This compound was reported to be a potent inactivator of ornithine decarboxylase [27] and as shown in Table II our results confirm this with liver and prostate extracts. α-Aminoadipate and α-ketoadipate did not inhibit ornithine decarboxylase activity. These results, therefore, suggest that rat ornithine decarboxylase could be responsible for the production of cadaverine observed in these experiments.

TABLE II

EFFECT OF VARIOUS INHIBITORS ON DECARBOXYLATION OF LYSINE AND ORNITHINE BY RAT LIVER EXTRACTS

The activity for each tissue in the absence of inhibitors was designated 100% and the percentage of this activity in the presence of the inhibitors is shown in parenthesis. n.d., not determined.

Source of extract	Inhibitor	Ornithine decarboxylase activity (pmol $^{14}CO_2$/mg protein per h)	Lysine decarboxylase activity (pmol $^{14}CO_2$/mg protein per h)	Cadaverine production (pmol/mg protein per h)
Liver	None	22 (100)	21 (100)	n.d.
	1 mM α-aminoadipate	21 (95)	1 (5)	n.d.
	1 mM α-ketoadipate	22 (100)	<1 (2)	n.d.
	0.1 mM α-difluoromethylornithine	1 (5)	19 (93)	n.d.
Regenerating liver	None	1750 (100)	36 (100)	18 (100)
	1 mM α-aminoadipate	1630 (93)	16 (44)	n.d.
	1 mM α-ketoadipate	1710 (98)	15 (40)	17 (97)
	0.1 mM α-difluoromethylornithine	51 (3)	19 (53)	1 (6)
Prostate	None	1720 (100)	20	19 (100)
	1 mM α-aminoadipate	1695 (99)	19 (93)	n.d.
	1 mM α-ketoadipate	1680 (98)	18 (88)	17 (95)
	0.1 mM α-difluoromethylornithine	67 (4)	1 (5)	<1 (3)

While these experiments were in progress, Persson [15] suggested that cadaverine production in androgen-stimulated mouse kidney may be mediated via the action of ornithine decarboxylase. As shown in Table III, we were able to confirm the previous reports [14,15] that cadaverine was formed by a lysine decarboxylase activity in extracts from kidneys from androgen-treated mice. Cadaverine formation was not inhibited by 1 mM α-ketoadipate, but was strongly reduced by α-difluoromethylornithine. Activity in producing $^{14}CO_2$ from [1-^{14}C]lysine was very low in extracts from female mouse kidneys and this activity was inhibited by α-ketoadipate and as in rat liver and kidney is unlikely to represent a true lysine decarboxylase. Extracts from male mouse kidneys had substantially greater activities, but extracts from mice treated with excess androgens had activities more than 10-fold greater still and this activity did produce virtually stoichiometric amounts of CO_2 and cadaverine. Such androgen-treated mouse kidney extracts had extremely high L-ornithine decarboxylase activities, more than 30-times those found in regenerating or thioacetamide-treated rat liver (Table III).

More convincing evidence that lysine decarboxylase activity is associated with rat liver ornithine decarboxylase is given in Table IV. L-Ornithine decarboxylase was purified almost 8000-fold and at each step in the purification, the specific activity was measured with either 0.42 mM L-ornithine or 0.5 mM L-lysine as substrate. The preparation contained L-lysine decarboxylase activity at all stages of purification. Under our standard assay conditions, the activity with ornithine as substrate was 100-times that with lysine and constant ratio of ornithine to lysine decarboxylase activities over an 8000-fold purification argues strongly that the two activities are associated with the same enzyme. As shown in Fig. 1 and Table V, the K_m for L-ornithine was 0.09 mM and the V was 16.9 μmol of $^{14}CO_2$/h per mg protein. L-Lysine was a competitive inhibitor

TABLE III

DECARBOXYLATION OF LYSINE AND ORNITHINE AND PRODUCTION OF CADAVERINE BY MOUSE KIDNEY EXTRACTS

Mouse kidney extracts were prepared as described in the text and used as a source of enzyme. Results for decarboxylase activities in the absence of additions are shown as the mean ± S.D. for 4—6 separate estimations. Other details were as for Tables II and III. n.d., not determined.

Source of extract	Additions	Ornithine decarboxylase activity (nmol $^{14}CO_2$/mg protein per h)	Lysine decarboxylase activity (nmol $^{14}CO_2$/mg protein per h)	Cadaverine production (nmol/mg protein per h)
Female kidney	None	0.41 ± 0.08	0.03 ± 0.01	<0.01
	1 mM α-ketoadipate	0.43	0.005	n.d.
	1 mM α-difluoromethylornithine	0.01	0.03	n.d.
Male kidney	None	3.7 ± 0.4	0.09 ± 0.03	0.075
Androgen-treated male kidney	None	62.3 ± 15.4	0.95 ± 0.15	0.85
	1 mM α-ketoadipate	60.4	0.90	0.85
	1 mM α-difluoromethylornithine	0.9	0.04	0.01

TABLE IV

PURIFICATION OF RAT LIVER ORNITHINE DECARBOXYLASE

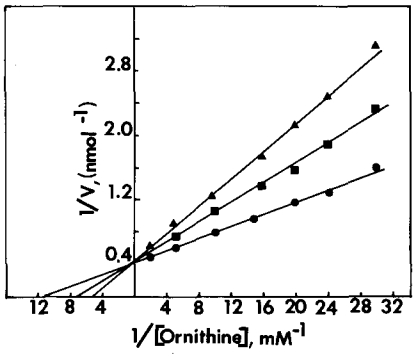
Activity was measured in the presence of 0.4 mM L-ornithine or 0.5 mM L-lysine. n.d., not determined.

Purification step	Total protein	Spec. act. (nmol $^{14}CO_2$/mg protein per h)		Ratio
		Ornithine decarboxylase	Lysine decarboxylase	
Crude extract	33 732	1.7 (100)	0.016 *	106
$(NH_4)_2SO_4$ fraction	10 353	4.8 (89)	n.d.	n.d.
DEAE-cellulose	310	135 (73)	1.35	100
Affinity chromatography	6	3 915 (41)	37	105
Sephadex chromatography	0.8	13 520 (19)	134	101

* Measured by cadaverine production.

of the release of $^{14}CO_2$ from [1-^{14}C]ornithine with a K_i of approximately 9 mM. When [1-^{14}C]lysine was the substrate, the K_m was also 9 mM and the V was 46 μmol $^{14}CO_2$/h per mg protein (Table V; Fig. 2). L-Ornithine was a competitive inhibitor of lysine decarboxylation having a K_i of 0.11 mM.

Certain other compounds were also tested for their effects on the decarboxylase. L-Arginine and L-histidine were not decarboxylated by the enzyme (the limit of detection was 1% of the rate with L-lysine when added at a concentration of 2 mM) and were not inhibitory to decarboxylation at a concentration of 20 mM with 0.4 mM ornithine as substrate. Putrescine inhibited ornithine decarboxylase activity competitively with a K_m of 1.4 mM, but cadaverine was much less inhibitory producing only 15% inhibition when added at a concentration of 25 mM in the presence of 0.1 mM ornithine. 1,3-Diaminopropane produced only a 10% inhibition under similar conditions. Spermidine was also



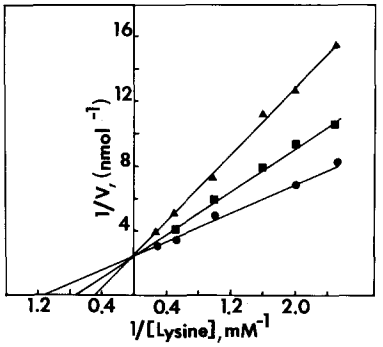


Fig. 1. Effect of L-lysine on decarboxylation of L-[1-^{14}C]ornithine by rat liver ornithine decarboxylase. Activity was measured over a 15 min incubation at 37°C using enzyme which had been preincubated for 5 min with the standard assay medium containing 0.04 mM pyridoxal phosphate and 1 mg/ml bovine serum albumin, but no substrate. Results are shown with no lysine (●), 5 mM (■) and 10 mM (▲) L-lysine.

Fig. 2. Effect of L-ornithine on decarboxylation of L-[1-^{14}C]lysine by rat liver ornithine decarboxylase. Activity was measured as described in Fig. 1 and results are shown with no ornithine (●), 0.05 mM L-ornithine (■) and 0.1 mM L-ornithine added (▲).

TABLE V

SUMMARY OF KINETIC PROPERTIES OF RAT LIVER ORNITHINE DECARBOXYLASE

Activity of the purified ornithine decarboxylase preparation was measured as described in Fig. 1 and 2.

Substance	K_m (mM)	K_i (mM)	V (μmol $^{14}CO_2$/mg per h)
L-Ornithine	0.09	—	16.9
L-Lysine	9.2	8.8	4.6
Putrescine	—	1.4	—
Cadaverine	—	>50	—
1,3-Diaminopropane	—	>50	—
D-Ornithine	—	7.1	—
Spermidine	—	5.5	—

a very weak competitive inhibitor having a K_i of 5.5 mM. D-Ornithine had a K_i of about 7.1 mM (Table V).

Another experiment showing that decarboxylation of lysine and ornithine appears to be carried out by the same enzyme is shown in Fig. 3 in which partially purified enzyme was incubated for 30 min in the presence of various concentrations of α-difluoromethylornithine. This compound leads to irreversible loss of ornithine decarboxylase activity. Aliquots of the enzyme were then assayed for ornithine and lysine decarboxylase activity. Both activities were lost to the same extent for each concentration of the irreversible inhibitor (Fig. 3).

Finally, it is known that rat liver ornithine decarboxylase activity is lost rapidly on inhibition of protein synthesis [4—6,9,10]. When rats pretreated 24 h earlier with thioacetamide as described for Table I were injected with cycloheximide (8 mg/kg body wt.), activity was lost with a half-life of 30 min which

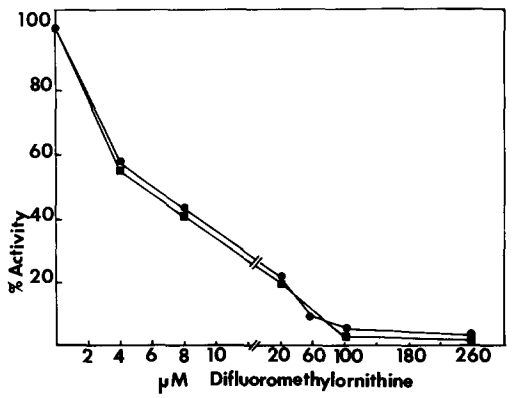


Fig. 3. Effect of incubation with α-difluoromethylornithine on ornithine and lysine decarboxylase activities. Rat liver ornithine decarboxylase from the DEAE-cellulose eluate was incubated for 15 min in the assay buffer without ornithine. Aliquots were then taken and assays of ornithine decarboxylase (•) or lysine decarboxylase (▪) activity was measured in an assay volume of 0.5 ml containing 0.1 mM L-[1-^{14}C]ornithine or 10 mM L-[U-^{14}C]lysine. The activity found in enzyme not exposed to the α-difluoromethylornithine was set at 100% for each substrate and the activities after exposure to the inhibitor concentration shown were expressed as a percentage of this.

is in agreement with estimates published by others [4—6,9,10]. The low activity of lysine decarboxylase prevented accurate estimation of the half-life for this activity, but 90% of the activity (measured by cadaverine production) was lost in 1 h after injection of the protein synthesis inhibitor. This finding is, therefore, in agreement with the concept that both activities reside in a single protein.

During the course of these experiments, we tested a number of crude tissue extracts for the presence of an arginine decarboxylase activity using 0.5 mM L-[U-^{14}C]arginine [10 Ci/mol] and the other assay conditions similar to those used for detection of the lysine decarboxylase activity. Some $^{14}CO_2$ production was observed with a number of tissues (brain, kidney, prostate, intestine and liver), whereas heart, skeletal muscle and spleen were not active. Liver was by far the most active tissue. However, the $^{14}CO_2$ production was not associated with the production of agmatine which was not detected in any assay. Instead, [^{14}C]putrescine was observed and the activity was drastically reduced by inclusion of unlabeled ornithine (1 mM) in the assays or by treatment of the extracts with 0.1 mM α-difluoromethylornithine. It, therefore, appears that all of the activity which was found is due to the conversion of some of the arginine to ornithine and subsequent decarboxylation of the ornithine. Since purified ornithine decarboxylase did not act on arginine, these results support our original report based on studies with the rat prostate that mammalian tissues do not contain any arginine decarboxylase activity [1].

Discussion

The present experiments indicate that rat tissues do contain a lysine decarboxylase activity capable of producing cadaverine, but indicate that all of this activity is due to the action of ornithine decarboxylase. The co-purification of lysine and ornithine decarboxylase activities, the parallel inactivation of both activities by α-difluoromethylornithine and loss of activity in response to cycloheximide, and the inhibition of lysine decarboxylase by ornithine with a K_i similar to the K_m for decarboxylation of ornithine (and vice versa) provide convincing evidence that the activity is derived from the same enzyme. Persson [15] has also concluded that mouse kidney ornithine decarboxylase acts on lysine. In both the present study and that of Persson [15], the K_m for lysine was an order of magnitude greater than the K_m for ornithine. For this reason, although the V with lysine was about 25% that with ornithine (Table V), the degree of lysine decarboxylation is likely to be small in most tissues. The results shown in Tables I and IV were carried out with substrate concentrations comparable to those found in vivo where in normal rat liver the ornithine concentration is about 0.5 mM [28] and the lysine concentration is about 0.8 mM [29]. At these concentrations, the ornithine decarboxylase activity is some 80% of the maximum, whereas the lysine decarboxylase activity is only 3% of the maximum. In addition, in vivo there would be substantial inhibition of the lysine-decarboxylating activity by the ornithine present. It is, therefore, understandable that the production of cadaverine and the lysine-decarboxylating activity has been detected previously [15] only in androgen-stimulated mouse kidneys which have very high ornithine decarboxylase activities and in

other mouse tissues in which very small amounts of cadaverine production were observed [16,17]. Many extrahepatic tissues contain significant levels of arginase which whilst less than those found in liver are more than sufficient to provide ornithine for polyamine synthesis [30–33]. However, it remains possible that in tissues other than liver which has a relatively high ornithine content, there might be significant decarboxylation of lysine to form cadaverine. In particular, the possibility that cells in culture which may be exposed to substantially higher concentrations of lysine than ornithine may produce cadaverine as well as putrescine appears to be worthy of consideration.

The present scheme for purifying rat liver ornithine decarboxylase produced in significantly better yield, a preparation of comparable specific activity to those previously reported [8–11]. We did not observe the multiple forms of the enzyme reported by Obenrader and Prouty [10] and the K_m for our enzyme preparation resembled that of their form II and was substantially less than for their form I. It is possible that these forms may represent partially degraded enzyme since ornithine decarboxylase is a very labile enzyme [1,6, 12]. Although the exact composition of the affinity adsorbent used in our purification is not clear, it permitted a 20-fold increase in specific activity to be achieved in a single step. Whilst our experiments were in progress, purification of mouse fibroblast ornithine decarboxylase by affinity chromatography on columns of pyridoxamine 5′-phosphate was reported. It appears, therefore, that the enzyme is bound by both affinity adducts, although they are not normal components in the enzyme reaction scheme [33–36]. Since the pyridoxamine absorbent produced a 500—fold purification [12], is somewhat quicker to prepare and likely to prove more stable, it would appear to be the method of choice in isolating the enzyme provided that its general applicability towards ornithine decarboxylases from other tissues can be established.

The present experiments are in agreement with those of Bey et al. [37] in indicating that the active center for ornithine decarboxylase strongly prefers the distance between the amino groups to be that of four carbon atoms. However, it is shown here five carbons can be accommodated with a reduced affinity. The inhibition of activity by putrescine which confirms earlier reports with less active preparations [1,7] is much stronger than the inhibition by cadaverine supporting this hypothesis. Previous studies of Heller et al. [33] are also in agreement with this concept. They found that *N*-(5′-phosphopyridoxyl)-lysine was inhibitory towards mammalian ornithine decarboxylase, but was less effective that *N*-(5′-phosphopyridoxyl)ornithine. Since 1,3-diaminopropane was even less inhibitory than cadaverine, it appears that when the amino groups are spaced by only three carbon atoms the molecule is too short for effective binding.

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References

1 Pegg, A.E. and Williams-Ashman, H.G. (1968) Biochem. J. 108, 533—539
2 Raina, A. and Jänne, J. (1968) Acta Chem. Scand. 22, 2375—2378
3 Tabor, H. and Tabor, C.W. (1972) Adv. Enzymol. 36, 203—268
4 Tabor, C.W. and Tabor, H. (1976) Annu. Rev. Biochem. 45, 285—306
5 Morris, D.R. and Fillingame, R.H. (1974) Annu. Rev. Biochem. 43, 303—325
6 Raina, A. and Jänne, J. (1975) Med. Biol. 53, 121—147
7 Jänne, J. and Williams-Ashman, H.G. (1971) J. Biol. Chem. 246, 1725—1732
8 Ono, M., Inoue, H., Suzuki, F. and Takeda, Y. (1972) Biochim. Biophys. Acta 284, 285—297
9 Hölttä, E. (1975) Biochim. Biophys. Acta 399, 420—427
10 Obenrader, M.F. and Prouty, W.F. (1977) J. Biol. Chem. 252, 2860—2865
11 Canellakis, E.S., Heller, J.S., Kyriakidis, D. and Chen, K.Y. (1978) Advances in Polyamine Research (Campbell, R.A., Morris, D.R., Bartos, D., Daves, G.D. and Bartos, F., eds.), Vol. 1, pp. 17—30, Raven Press, New York
12 Boucek, R.J. and Lembach, K.J. (1977) Arch. Biochem. Biophys. 184, 408—415
13 Smith, T.A. (1971) Biol. Rev. 46, 201—241
14 Henningson, S., Persson, L. and Rosengren, E. (1976) Acta Physiol. Scand. 98, 445—449
15 Persson, L. (1977) Acta Physiol. Scand. 100, 424—429
16 Schmidt-Glenewinkel, T., Nomura, Y. and Giacobini, E. (1977) Neurochem. Res. 2, 619—637
17 Stepita-Klauco, M. and Dolezalova, H. (1974) Nature 252, 158—159
18 Caldarera, C.M., Barbiroli, B. and Moruzzi, G. (1965) Biochem. J. 97, 84—88
19 Jänne, J., Pösö, H. and Raina, A. (1978) Biochim. Biophys. Acta 473, 241—293
20 Dion, A.S. and Cohen, S.S. (1972) J. Virol. 9, 423—429
21 Ikeda, S., Hara, H. and Saburo, F. (1974) Biochim. Biophys. Acta 372, 400—406
22 Ikeda, S., Hara, H., Sugimoto, S. and Fukui, S. (1975) FEBS Lett. 56, 307—311
23 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
24 Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406—4412
25 Grove, J.A., Linn, T.G., Willett, C.J. and Henderson, L.M. (1970) Biochim. Biophys. Acta 215, 191—194
26 Borsook, H., Deasy, C.L., Haagen-Smit, A.J., Keighley, G. and Lowy, P.H. (1948) J. Biol. Chem. 176, 1395—1400
27 Metcalf, B.W., Bey, P., Danzin, C., Jung, M.J., Casara, P. and Vevert, J.P. (1978) J. Am. Chem. Soc. 100, 2551—2553
28 Jefferson, L.S. and Pegg, A.E. (1977) Biochim. Biophys. Acta 484, 177—187
29 Tolman, E.L., Schworer, C.M. and Jefferson, L.S. (1975) J. Biol. Chem. 248, 4552—4560
30 Williams-Ashman, H.G., Pegg, A.E. and Lockwood, D.H. (1969) Adv. Enzyme Regul. 7, 291—323
31 Oka, T. and Perry, J.W. (1974) Nature 250, 660—661
32 Klein, D. and Morris, D.R. (1978) Biochem. Biophys. Res. Commun. 81, 199—204
33 Heller, J.S., Canellakis, E.S., Bussolotti, D.L. and Coward, J.K. (1975) Biochim. Biophys. Acta 403, 197—207
34 Clark, J.L. and Fuller, J.L. (1976) Eur. J. Biochem. 67, 303—314
35 O'Leary, M.H. and Baughn, R.L. (1977) J. Biol. Chem. 252, 7168—7173
36 O'Leary, M.H. and Herreid, R.M. (1978) Biochemistry 17, 1010—1014
37 Bey, P., Danzin, C., Van Dorsellaer, V., Mamont, P., Jung, M. and Tardif, C. (1978) J. Med. Chem. 21, 50—55