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Occurrence of Ornithine Decarboxylase and Polyamines in Cartilage

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The activity of ornithine decarboxylase was investigated in cartilage from chick embryos, rabbits, rats and human foetuses. The enzyme activity in these cartilages was of the same order as that detected in other body tissues. Ornithine decarboxylase activity in chick-embryo cartilage and liver was the same when compared on the basis of total soluble tissue protein. The cartilage enzyme exhibited a pH optimum of 6.5 and a K_m for ornithine of 0.16mM. Ornithine decarboxylase activity in chick-embryo pelvic leaflets was maintained at the value *in vivo* for up to 22h when the isolated tissue was incubated in a modified Waymouth's medium (MB 752/1) at 37°C. After addition of cycloheximide to the incubation medium, ornithine decarboxylase activity declined, with a half-life of 40min. The concentrations of the polyamines spermidine and spermine in chick-embryo pelvic cartilage and rabbit costal cartilage were of the same order as the concentrations detected in other tissues.

The polyamines putrescine, spermidine and spermine have been detected at up to millimolar concentrations in many animal tissues as well as in plants, prokaryotes and certain bacteriophages (Tabor & Tabor, 1964). The intracellular concentrations of these polycations rise in response to various stimuli, including those causing cell proliferation and hypertrophy (Jänne, 1967), and correlate well with cell growth (Heby *et al.*, 1975). It has been proposed that an increased polyamine concentration is an essential part of the cellular pleiotypic response to a growth stimulus, but the precise function of these intracellular polycations has not been resolved (Raina & Jänne, 1975). The first and probably rate-limiting enzyme in the biosynthesis of polyamines is ornithine decarboxylase (EC 4.1.1.17). The activity of this enzyme is raised in a number of tissues after growth-hormone administration (Sogani *et al.*, 1972). Cartilage in young animals contains a high proportion of proliferating and hypertrophic cells and is also a main target tissue for growth hormone. We therefore decided to investigate whether significant ornithine decarboxylase activity exists in cartilage and also to measure the tissue concentrations of polyamines.

Experimental

Tissue

Chick embryos, days 11–14, were of the White Leghorn strain. Dutch White rabbits were 4–7 weeks old, and rats, 3–4 weeks old, were male Sprague–Dawley animals. Human tissue was obtained from therapeutic abortions.

Assay of ornithine decarboxylase

The ornithine decarboxylase assay was slightly modified from that of Russell & Snyder (1968). Tissue was quickly removed from the animal into ice-cold 0.9% NaCl and assayed immediately without further storage, care being taken to perform all steps at close to 0°C up until the final incubation. Tissue was homogenized (Ultra-Turrax) in 10vol. of buffer containing 0.5M-KH_2PO_4, 5mM-dithiothreitol, and 0.5mM-pyridoxal phosphate and adjusted to pH6.5 with 1.0M-NaOH. After centrifugation at 15000*g* for 30min, samples of the supernatant were made up to a volume of 2.0ml with the above buffer and incubated at 37°C for 30min with 1.0μmol of L-ornithine and 0.5μCi of DL-[1-^{14}C]ornithine in closed Universal 30ml bottles (supplied by Sterilin, Richmond, Surrey, U.K.).

The reaction was stopped by injection of 2ml of 50% (w/v) citric acid and released $^{14}CO_2$ was captured in 0.3ml of 1M-Hyamine hydroxide in a glass container (0.6cm×4.0cm), which was included within the Universal bottle. After addition of 4.0ml of a toluene scintillator (0.4% 2,5-diphenyloxazole) to the glass insert, the absorbed radioactivity was counted in a Packard liquid-scintillation spectrometer (counting efficiency 85%). Units of activity were normally expressed as nmol of CO_2 released/30min per mg of tissue (wet wt.).

When activity was presented on a protein basis, the protein concentration of the 15000*g* supernatant was measured (Lowry *et al.*, 1951) with bovine serum albumin as standard. As dithiothreitol interferes in this determination, it was removed by precipitation of

the protein with 10% (w/v) trichloroacetic acid; the protein was sedimented, washed with 5% trichloroacetic acid and redissolved in the alkaline Lowry *et al.* (1951) reagent. DNA was measured in the unfractionated tissue homogenate by the method of Croft & Lubran (1965). For an estimation of total cell count in liver and cartilage, the tissues were digested overnight at 37°C in Waymouth's medium MB 752/1, containing 0.05% collagenase and 0.25% trypsin. The resulting cell suspension was counted with a haemocytometer slide.

Ornithine decarboxylase was also assayed by measuring the production of putrescine from L-[G-^{3}H]ornithine after separation of the former by t.l.c. A tissue extract was prepared and incubated as described above, by using [G-^{3}H]ornithine (200μCi/ml) instead of [1-^{14}C]ornithine. After incubation, ethanol was added to a final concentration of 90% (w/v); mixtures were left overnight at 4°C and centrifuged at 3000*g* for 30min. The supernatant was evaporated and the residue dissolved in 0.2ml of water. Samples (1, 2 or 5μl) of the resulting solution, together with ornithine and putrescine standards, were chromatographed on thin-layer plates of cellulose (0.1mm thickness) in a solvent of pyridine/acetone/aq. NH_3 (35%, w/v)/water (10:6:1:4, by vol.). The resulting positions of ornithine and putrescine were located with a ninhydrin spray. Ornithine and putrescine had R_F values of 0.18 and 0.75 respectively in this system, which also separated spermine and spermidine (R_F values 0.68 and 0.59 respectively). Radioactivity of the separated ornithine and putrescine was determined with a scanning gas-flow counter.

Measurement of tissue polyamine contents

Polyamines were extracted from tissues into alkaline butanol as described by Raina (1963). Putrescine, spermidine and spermine were then separated by high-voltage electrophoresis at room temperature (21°C) at 50V/cm, for 65min in 0.1M-sodium citrate buffer, pH3.5. The separated polyamines were quantified with cadmium/ninhydrin reagent (Heilmann *et al.*, 1957).

Chemicals

DL-[1-^{14}C]Ornithine (10–30mCi/mmol) was from The Radiochemical Centre, Amersham, Bucks., U.K.; L-[G-^{3}H]ornithine (2.5Ci/mmol) was from New England Nuclear, Boston, MA, U.S.A.; Waymouth's medium WB 752/1 was from Flow Laboratories, Irvine, Scotland, U.K.; dithiothreitol, pyridoxal phosphate, Hyamine hydroxide, collagenase, trypsin and Hepes [2-(*N*-2-hydroxymethylpiperazin-*N'*-yl)ethanesulphonic acid] buffer were from Sigma (London) Chemical Co., London S.W.6, U.K. All other chemicals were of analytical grade.

Results

Ornithine decarboxylase in cartilage

Ornithine decarboxylase was assayed in the cartilage of several species by measuring the production of $^{14}CO_2$ from L-[1-^{14}C]ornithine after incubation with tissue extract. The assay was linear with respect to both time of incubation and tissue concentration used. Enzyme activities were unaltered after dialysis against the homogenization buffer, indicating a lack of significant effect of endogenous tissue ornithine on the specific radioactivity of the added [^{14}C]ornithine. Ornithine decarboxylase was detected in cartilage from chick embryos, human foetuses, rabbits and rats at an activity similar to that occurring in other tissues (Table 1). Rat costal cartilage contained the lowest detected activity, and no activity was found in bone. Rabbit articular cartilage (knee and shoulder joints) contained slightly more activity than did costal cartilage. A more detailed comparison of ornithine decarboxylase activities in chick-embryo liver and cartilage was performed by calculating the tissue activities on a protein, DNA and tissue-cell-count basis (Table 2). Both liver and cartilage contained approximately the same amount of ornithine decarboxylase on the basis of total soluble protein in the tissues.

Ornithine decarboxylase activity of chick cartilage was also measured by incubating tissue extract with [G-^{3}H]ornithine and measuring the production of putrescine by using t.l.c. to separate the latter. The two assays together revealed that the production of

Table 1. *Comparison of tissue ornithine decarboxylase contents*

Activity was measured as the release of $^{14}CO_2$ from [1-^{14}C]ornithine after incubation with tissue extract. Results are means ± S.D. for the numbers of observations in parentheses.

Animal	Tissue	Ornithine decarboxylase activity (nmol of CO_2 released/30min per g of tissue)
Chick embryo (11–14 days)	Pelvic cartilage	3.0 ± 0.3 (12)
	Liver	17.4 ± 1.5 (10)
Human foetus (25 weeks)	Costal cartilage	1.4 ± 0.5 (2)
	Skin	0.8 ± 0.1 (2)
	Rib bone	<0.1
Rabbit (4–6 weeks)	Costal cartilage	5.2 ± 1.5 (9)
	Articular cartilage	8.1 ± 1.3 (4)
	Skeletal muscle	3.7 ± 2.9 (4)
	Kidney	15.1 ± 1.9 (4)
	Liver	26.9 ± 11.3 (4)
Rat (3–4 weeks)	Costal cartilage	0.4 ± 0.1 (5)
	Liver	7.8 ± 2.2 (5)

Table 2. *Comparison of ornithine decarboxylase activity in liver and pelvic cartilage of day-11–14 chick embryos on the basis of tissue wet weight, total soluble protein, total DNA and tissue cell count*

Results are means ± S.D. for the numbers of observations in parentheses.

Units of activity	Liver	Cartilage
nmol of CO_2/30min per g of tissue	17.4±1.5 (10)	3.0±0.3 (12)
nmol of CO_2/30min per mg of protein	1.0	0.9
nmol of CO_2/30min per mg of DNA	8.6	4.4
nmol of CO_2/30min per 10^8 cells	5.9	1.2

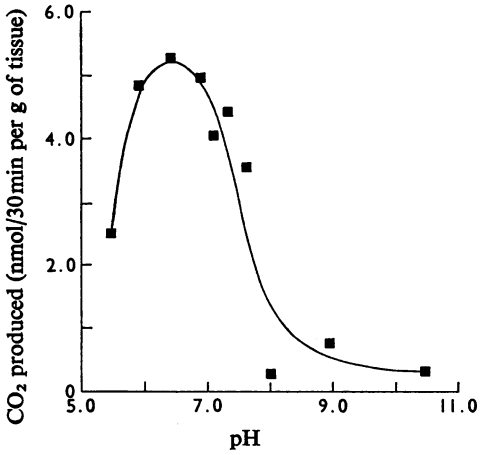

Fig. 1. *Variation of ornithine decarboxylase activity with pH in an extract of chick-embryo pelvic cartilage*
For experimental details see the text.

$^{14}CO_2$ from [1-^{14}C]ornithine was approximately stoicheiometric with putrescine formation.

Ornithine decarboxylase activity in cartilage exhibited a distinct maximum of activity at pH 6.5 (Fig. 1). An analysis of the variation of enzyme activity with ornithine concentration revealed that the K_m values for the liver and cartilage enzymes were 0.16 and 0.26mM respectively (Fig. 2), the difference not being statistically significant.

Chick-embryo pelvic leaflets were incubated *in vitro* in Waymouth's medium MB 752/1, containing added Hepes buffer (20mM), serine (1.8mM) and glutamine (3.5mM). Under these conditions ornithine decarboxylase activity was maintained at the value *in vivo* for up to 22h at 37°C. Addition of cycloheximide (100μg/ml) to the incubation medium resulted in the enzyme activity declining, with a half-life of 40min (Fig. 3).

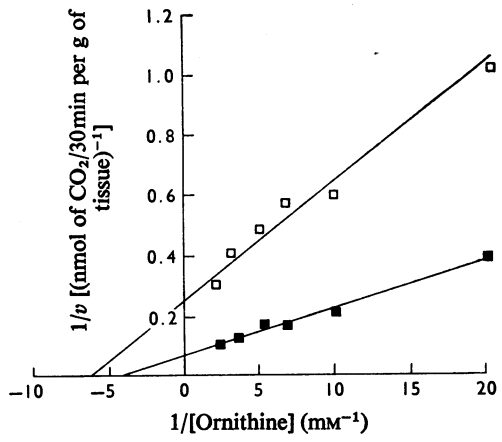

Fig. 2. *Lineweaver–Burk plot for dependence of ornithine decarboxylase activity on ornithine concentration*
Extracts of chick-embryo cartilage (□) and liver (■) were prepared and ornithine decarboxylase activity (v) was assayed by measuring production of $^{14}CO_2$ from DL-[1-^{14}C]ornithine as described in the text.

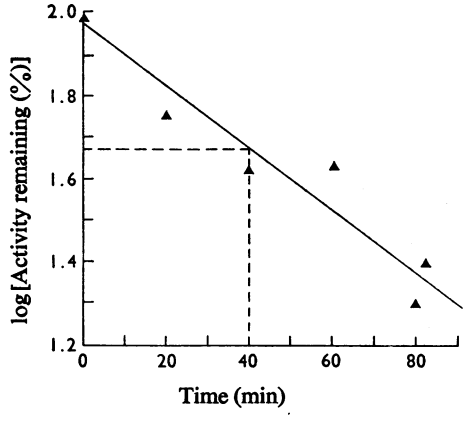

Fig. 3. *Decay of ornithine decarboxylase activity in chick-embryo pelvic-cartilage leaflets incubated in vitro with 100μg of cycloheximide/ml*
For experimental details see the text. Each point is the mean of four observations. ----- indicates the half-life of enzyme activity

Polyamines in cartilage

Polyamines were isolated and quantified from the chick liver and cartilage and from rabbit cartilage, liver, skeletal muscle and heart (Table 3). On a basis of tissue wet weight, chick cartilage contained only slightly less spermidine and spermine than did liver, spermine predominating in cartilage. Cartilage from 5–6-week-old rabbits contained similar polyamine concentrations to those found in muscle and brain of

Table 3. *Amounts of spermidine and spermine in tissue of 5–7-week-old rabbits and day-11–14 chick embryos*
Results are presented as μmol/g of tissue (wet wt.). Results were obtained with pooled tissue from the number of animals indicated in parentheses.

Animal	Tissue	Amount (μmol/g of tissue) Spermidine	Spermine
Rabbit	Costal cartilage (4)	0.09	0.12
	Heart (2)	0.15	0.14
	Brain (1)	0.29	0.20
	Skeletal muscle (3)	0.04	0.20
	Liver (2)	0.43	0.55
Chick embryo	Pelvic cartilage (48)	0.29	0.36
	Liver (48)	0.57	0.43

the same animals. In cartilage, as in most tissues examined, putrescine concentrations were below the limits of sensitivity of the system.

Discussion

We have demonstrated that ornithine decarboxylase activity and the content of the polyamines spermidine and spermine in cartilage are similar to those found in other tissues.

The properties described for the enzyme are similar to those reported for the enzyme purified from rat prostate gland (Jänne & Williams-Ashman, 1971). The extremely short half-life of 40 min is consistent with ornithine decarboxylase having an important regulatory role in chondrocyte metabolism. The amounts of both spermidine and spermine detected in chick cartilage can be converted into a concentration of approx. 0.4 mM if divided by the water content of the tissue, assuming free distribution of the polyamines. This is similar to or higher than the tissue concentration of most free amino acids (Munro & Fleck, 1969).

As has been generally found in other animal tissues, as distinct from bacteria (Tabor & Tabor, 1964), putrescine if present was there in quantities much less than spermidine and spermine. As the only known biosynthetic sequence for spermidine and spermine in animal tissues flows through putrescine, the latter diamine presumably has a much shorter half-life than the two polyamines into which it is converted. A diamine oxidase in animal tissues (Blaschko, 1974) may contribute to this turnover.

The function of these relatively high concentrations of intracellular polycations has not yet been fully defined. They appear to be involved in the cell growth process and may be important in binding intracellular acids such as DNA, RNA and membrane phospholipids. The polyamines may be involved in such a capacity in cartilage as well as in binding the high concentration of acidic proteoglycans found in this tissue. Certainly the activity of ornithine decarboxylase and amounts of polyamines found in cartilage imply a significant role for them in the tissue metabolism.

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