Br. J. Pharmac. (1971), **41**, 294–301.

Decarboxylases of histidine and ornithine in chick embryo

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Summary

1. The activities of histidine and ornithine decarboxylases as well as the histamine content of the developing chick embryo were studied.

2. Histidine decarboxylase (L-histidine carboxy-lyase ; E.C. 4.1.1.22) activity was fairly low with a tendency to increase at later stages of development. This enzyme was preferentially present in the supernatant fraction of the tissue homogenate. α-Methyl-histidine, but not α-methyl-DOPA, inhibited its activity.

3. The histamine content per gramme of embryo was low with a tendency to increase with the age of the embryo.

4. Ornithine decarboxylase (L-ornithine carboxy-lyase ; E.C. 4.1.1.17) activity was high at the beginning of the stages of development investigated, but later there was a steep fall in activity. A noticeable feature was that while the activity in the residual fraction of the homogenate remained almost constant during development, the activity in the supernatant fraction was high in the early stages, then fell rapidly to nearly zero at later stages.

Introduction

Based upon experimental evidence from foetal rat tissue, workers from this laboratory suggested in 1960 that there is some relationship between the histamine synthesizing ability (histamine forming capacity, HFC) of certain tissues and rapid growth (Kahlson & Rosengren, 1968). In all instances where the phenomenon was demonstrated, the high HFC was directly referable to a striking increase in histidine decarboxylase activity. The contribution to tissue histamine synthesis by DOPA decarboxylase was found to be practically negligible.

To strengthen the proposition, Grahn, Hughes, Kahlson & Rosengren (1969), and Grahn & Rosengren (1970) demonstrated that α-methyl-histidine, a specific *in vivo* and *in vitro* inhibitor of histidine decarboxylase (Kahlson, Rosengren & Thunberg, 1963) retarded protein synthesis.

The view that high HFC may be essential for certain types of rapid growth appeared to have found support also from the rarely quoted report of Misrahy (1946) that increases occurred in histamine and adenosine concentrations in the developing chick embryo. Like histamine, adenine is formed in certain organisms in the course of histidine metabolism (Sprinson & Rittenberg, 1952 ; Moyed & Magasanik, 1960 ; Shedlovsky & Magasanik, 1962a, b).

Russell & Snyder (1968) investigated the level of activity of six amino-acid decarboxylases in various rapidly growing tissues and found high histidine decarboxylase activity in some of them. In other tissues, especially in the chick embryo, these authors noted very low histidine decarboxylase activity (no figures given) and found high ornithine decarboxylase activity. This is in accord with Raina (1963) and with Caldarera, Barbiroli & Moruzzi (1965) who studied the formation of polyamines in the developing chick embryo.

The aims of the present investigation were to define the pattern of the two enzymic activities and to establish the hitherto uninvestigated disposition of the enzymes and any possible changes therein during the course of development of the embryo.

Methods

Tissue preparations

Chick embryos at various developmental stages were obtained from hens' eggs incubated at 37·8–40·6° C, humidity about 60%. Material obtained from the seventh day of development and until hatching was investigated. The embryo was separated from the membranes. Formation of histamine was determined by the 'pipsyl' method (see below) in minced tissues; all other enzyme assays were carried out on homogenized tissue and its subfractions.

Chemicals

DL-ornithine-1-^{14}C monohydrochloride (2·74 or 3·31 mCi/mM) was obtained from New England Nuclear Corp. U.S.A.; L-histidine carboxyl ^{14}C (4 mCi/mM) was bought from Calbiochem, U.S.A.; 2-ring labelled histidine ^{14}C (58·3 mCi/mM) was purchased from the Radiochemical centre, Amersham, England; DL-α-methyl-histidine and DL-α-methyl-DOPA were generous gifts from Merck, Rahway, New Jersey; pyridoxal-5-phosphate was obtained from Sigma Chemical Co. Ltd., U.S.A.

Preparation of enzymes for determining activity and localization

Whole embryos were homogenized with three volumes (w/v) of cold 0·1 M sodium phosphate buffer, pH 7·4 at 0–4° C. Unless otherwise stated, all homogenizations and centrifugations were done at 0–4° C. The homogenate was filtered through gauze to remove coarse material. Part of the filtrate (referred to henceforth simply as homogenate) was centrifuged at 20,000 *g* for 20 min in a refrigerated MSE superspeed 50 centrifuge. The supernatant fraction was carefully decanted and kept in the cold for assay. The residue (particulate fraction) was washed three times with a third of the original volume of buffer, resuspended in buffer, rehomogenized and kept in the cold for assay.

Determination of enzyme activity

CO_2 method. This method, originally devised by Kobayashi (1963), modified by Levine & Watts (1966) and elaborated by Grahn & Rosengren (1968) for use in our laboratory, was used to determine decarboxylation of histidine and ornithine in the homogenate, supernatant and residual tissue fractions. The reaction mixture

contained 0·1 μmol pyridoxal phosphate, 0·8–1·0 ml enzyme preparation, either 0·5 μCi carboxyl labelled ornithine (0·46 mM final conc.) or 1.25 μCi carboxyl labelled histidine (0·1 mM final conc.), and phosphate buffer to make a final volume of 2·0 ml. The reaction mixture, excluding substrate, was preincubated for 5 min at 37° C with mechanical shaking. After adding substrate, the incubation proper was carried out for 60 min at 37° C and with shaking. The reaction was stopped by tipping 1 ml of 2 M citric acid from the side arm into the reaction mixture whereby any CO_2 was expelled. The CO_2 was trapped on specially prepared Munktells filter paper saturated with 100 μl hydroxide of hyamine 10-X. After shaking for another hour at 37° C to ensure complete CO_2 absorption, the filter paper was dropped into Bray (1960) scintillation mixture for counting in either a Packard Tricarb spectrophotometer or a Nuclear Chicago spectrophotometer.

'Pipsyl' method. Besides assays with the CO_2 method, histidine decarboxylase activity (histamine forming capacity, HFC) was determined by the 'pipsyl' method of Schayer (1956) as adapted for use in our laboratory (Kahlson *et al.*, 1963). Determinations with this method were done in one type of enzyme preparation only, in carefully minced tissue, involving incubation with 2-ring-labelled L-histidine (0·08 mM final conc.).

Measurement of protein concentration

The protein concentrations of the enzyme preparations were determined by the method of Lowry, Rosenbrough, Farr & Randall (1951).

Results

It should be mentioned that the duration of embryonic development in the chick is about 21 days. The values for the determinations of the decarboxylases in the very early days of the development of the embryo are not included as it was difficult to separate the embryo from the membranes and as sufficient amount of tissue could not be collected.

HFC and histamine content

Histidine decarboxylase activity of the developing chick embryo was low and remained low even until hatching as judged by the determination of CO_2 evolution (Fig. 1) and by the HFC determination by the 'pipsyl' method (Table 1). The histamine content of the embryo was also low during the entire period of development. Table I shows that at the eighteenth–twentieth day there was a tendency for the HFC and the histamine content values to increase.

The antihistaminic compound mepyramine did not in all instances abolish the contraction produced by the extract on a segment of guinea-pig gut (Table 1). This observation indicates the occasional occurrence in the extract of gut contracting agent(s) other than histamine.

The topical distribution of histidine decarboxylase was investigated in one embryo at the nineteenth day using the 'pipsyl' method. This experiment revealed a differential distribution of HFC. The values obtained for skin, muscle with bones, liver and pooled heart, lung and spleen, were respectively: 76, 92, 44, 36 ng/g.

The corresponding figures for histamine content were: 2·2, 3·3, 1·6, 1·6 μg/g.

Histidine decarboxylase was localized mainly in the supernatant, in contrast to ornithine decarboxylase, as will be described.

Effect of α-methyl-histidine and α-methyl-DOPA on histidine decarboxylase

The nature of the histamine forming enzyme was investigated by determining the inhibition produced by, respectively, α-methyl-histidine and α-methyl-DOPA. The former compound strongly inhibits histidine decarboxylase whereas the latter does not significantly inhibit this enzyme (Kahlson & Rosengren, 1968). The present determinations were carried out with the 'pipsyl' method. α-Methyl-histidine in a final concentration of 2·5 mM inhibited the decarboxylation of labelled histidine incubated with chick embryo tissue aged 12–20 days (Table 2). Unlike α-methyl

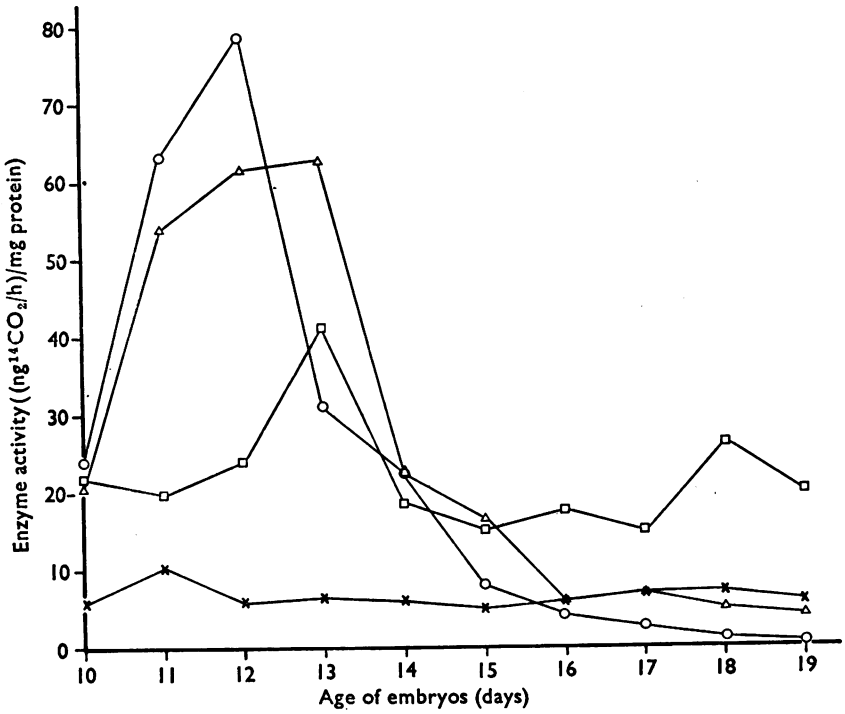

FIG. 1. Ornithine decarboxylase activity in the developing chick embryo. Ornithine decarboxylase: homogenate, △——△; supernatant, ○——○; residue, □——□. Histidine decarboxylase (supernatant only), ×——×.

TABLE 1. *Histamine forming capacity (HFC) and histamine content of the developing whole chick embryo*

Days of incubation	7	8	9	10	11	12	13	14	15	16	17	18	19	20
HFC	–	12	12			23	–	9	8	15	10	49	26	33
ng/g		–					–	–	–	9	14	33		39
Histamine	1·1	0·8	0·6			0·6	0·2	4·7*	0·7	1·3*	0·3	2·4	3·0	2·3
content μg/g		0·5					0·9	0·2	1·3	1·3	0·5	1·8*		2·2

Each figure represents the means of two determinations using pooled minced embryos. HFC is expressed as ng/g and histamine content (determined as described by Code, 1937) as μg (base)/g.
* Effect of extract on the gut only partially abolished by mepyramine.

histidine, α-methyl-DOPA (final concentration 2·5 mM) was inhibitory only in one experiment (Table 2), indicating that DOPA-decarboxylase activity did not noticeably contribute to the formation of histamine.

Ornithine decarboxylase activity

Determinations were made by the CO_2 method. The source of the enzyme, the homogenate, the supernatant and the residue were as described under **Methods**. The enzyme activity rose steeply from the tenth day onwards to attain peak values on the twelfth–thirteenth day, whereafter the activity declined precipitously (Fig. 1).

Regarding the localization of ornithine decarboxylase, changes occurred in the ratio between the two fractions investigated. On the tenth–twelfth days more enzyme was present in the supernatant than in the particulate fraction. On the fourteenth day, approximately equal activities were detected in both fractions but from the fifteenth day of incubation onwards, more enzyme activity was demonstrable in the particulate fraction than in the supernatant. In other words, the activity in the residue appeared to remain almost constant throughout all the stages examined, while it declined in the supernatant. Especially noticeable in this respect were the results obtained during the last days of development when the activity in the supernatant steadily declined but remained almost unchanged in the residue. This is shown in Fig. 1 and pertinent values are given in Table 3.

Discussion

For the first time, the present study gives figures for the histidine decarboxylase activity of the developing chick embryo. The activity of this enzyme showed a

TABLE 2. *Effects of α-methyl-histidine and α-methyl-DOPA on the histidine decarboxylase activity of the developing chick embryo*

Days of incubation	Histamine forming capacity				
	Control	α-Methyl-histidine		α-Methyl-DOPA	
	ng/g	ng/g	% of control	ng/g	% of control
12	23	—	—	24	104
15	9	9	100	10	111
16	9	6	67	9	100
17	12	8	67	16	133
18	41	16	39	20	49
19	26	12	46	27	104
20	36	16	45	29	81

Each figure represents the means of at least two determinations.

TABLE 3. *Changes in the distribution of chick embryo ornithine decarboxylase activity during growth*

Age of embryo (days)	Enzyme fraction	Total protein (corrected for losses) (mg)	Specific activity (units)	Total activity (total units)	% Distribution of activity
11*	Homogenate	187	33	6,084	100
	Supernatant	115	40	4,570	75
	Residue	72	20	1,440	24
19+	Homogenate	828	5	3,726	100
	Supernatant	676	1	676	18
	Residue	152	20	3,040	82

* Mean of three pooled embryos assayed in triplicate ; + mean of triplicate assays of a single embryo. Specific activity (units of enzyme) is defined as (ng ornithine destroyed/mg protein)/hour.

tendency to increase with the age of the embryo when determined by the 'pipsyl' method which is more reliable than the CO_2 method (Grahn & Rosengren, 1968). The present figures, around 40 ng/g in the chick embryo, should be seen in relation to HFC values in some other mammalian tissues; for example, 20 ng/g in the hamster and cat foetus, 40 ng/g in feline and human gastric mucosa, 20 ng/g in rat skin. Thus, in the chick embryo, histidine decarboxylase activity and histamine formation are relatively high. Judged from the single experiment done, there is a differential tissue distribution of chick embryo HFC, a feature which pertains also to some mammalian foetuses. Chick embryo histidine decarboxylase was inhibited by α-methylhistidine, but not by α-methyl-DOPA, which indicates similarity of the enzyme with mammalian foetal histidine decarboxylase (Kahlson & Rosengren, 1968). The physiological significance of chick histidine decarboxylase would be better understood if the enzyme could be inhibited *in vivo*.

Investigations so far carried out have shown that the histamine content in embryonic and rapidly growing tissue is mostly very low, even in instances where the HFC is very high. This is presumably due to rapid degradation of the histamine formed in these tissues. In fact, the discrepancy between HFC and histamine content is one of the characteristics of histamine metabolism in various embryonic and rapidly growing tissues. The chick embryo develops within a closed shell from which histamine formed cannot be drained as in mammalian foetuses. In the present study the histamine content at no stage attained 4 μg/g. Misrahy (1946) reported a continuous increase in histamine per gramme of embryo, from about 0·1 μg(base)/g to 0·9 μg during the eleventh–nineteenth day of development. These figures are somewhat lower than in the present study, but they agree with the present ones in the tendency towards a continuous increase. In the present experiments the occurrence of a non-histamine component contracting the gut was noted occasionally (Table 1).

The catabolism of histamine in the chick embryo has not been investigated. The increase in histamine content during the days before hatching when the HFC has a tendency to increase would imply the absence, or relative ineffectiveness, of enzymes catabolizing the histamine continuously formed.

The occurrence of ornithine decarboxylase in the supernatant fraction of various tissues has been reported in the rat prostate gland (Pegg & Williams-Ashman, 1968) and in rat tissues and chick embryo (Russell & Snyder, 1968). In the experiments by the latter authors a sharp peak in ornithine decarboxylase occurred at the fourth–sixth day, whereupon the activity progressively declined, whereas in the present study the peak was recorded at a considerably later stage of development of the chick embryo, the eleventh– thirteenth day. As seen in Fig. 1, ornithine decarboxylase was determined from the tenth to the nineteenth day, because it was assumed that changes occurring at this later stage of development would be of particular significance and because of the difficulty in obtaining sufficient amounts of proper tissue from a tiny embryo. The inconsistency in results pertaining to the peak at the late stage is presumably due to differences in experimental procedures or to a strain difference.

It appears that Russell & Snyder (1968) investigated only the supernatants, and not the differential distribution of the enzyme, the study of which has yielded new information. The present demonstration that in the course of development ornithine decarboxylase activity in the residue is fairly constant with a concurrent steady

decrease in the supernatant fraction (Fig. 1 and Table 3), raises the question as to the implication of this observation. It could mean that the site of the synthesis of the enzyme in the chick embryo varies with the stages of development. The alternative that there might be an unknown natural inhibitor of the enzyme, which being soluble, was fractionated along with the supernatant by the centrifugation step was ruled out by assaying the supernatant after dialysis for 6 h against 0·01 M buffer without observing any increase in the activity. Any suggestion, however, that the high activity in the residue at later stages of development could be attributed to contamination by the supernatant fraction is discounted because enzyme activity in the supernatant itself was very low. A fourth possibility that the observed changes might have been caused by the presence of chick embryo ornithine decarboxylase isoenzymes was not investigated. From the present results it would appear that studies on ornithine decarboxylase of chick embryo, and perhaps also of other tissues, concerned exclusively with a single fraction of the homogenate may not be fully representative of the changes in the enzyme activity occurring during growth.

The product of ornithine decarboxylase activity is putrescine which like histamine is a diamine. Raina & Jänne (1970) found increased ornithine decarboxylase activity in the regenerating rat liver after partial hepatectomy. Russell & Snyder (1968) confirmed the results by Raina & Jänne, and in addition reported high ornithine decarboxylase activity in the STAT-1 rat sarcoma and the chick embryo. Assuming that in certain rapidly growing tissues putrescine plays a role similar to that assumed for histamine, Kahlson & Rosengren (1970) suggested that the original hypothesis regarding HFC and tissue growth be broadened to state that a high rate of intracellular diamine formation is associated with and presumably essential to certain types of rapid tissue growth. Attempts to inhibit histamine formation *in vivo* have proceeded since 1960; the discovery of means to inhibit ornithine decarboxylase *in vivo* would perhaps disclose whether this enzyme is causally associated with tissue growth and development.

We are indebted to Professor Adam Deutsch, Institute of Biochemistry, University of Lund, and to Professor Georg Kahlson of this Institute for their interest in this work. This work was supported by a grant from the Swedish Cancer Research Council (Grant No 268-K70-01X).

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(*Received August* 18, 1970)