DL-α-Difluoromethylornithine, an Enzyme-Activated Irreversible Inhibitor of Ornithine Decarboxylase, Blocks Chick Embryo Development at Gastrulation *

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In developing invertebrate embryos there is a requirement for polyamines during gastrulation,[1] the developmental process that delineates the inception of embryonic autonomy. The discovery of this polyamine dependence was made following the production of specific inhibitors of the polyamine biosynthetic enzymes. The initial enzyme in the synthesis of the polyamines (putrescine, spermidine and spermine) is ornithine decarboxylase (ODC; L-ornithine carboxy-lyase, EC 4.1.1.17), which catalyzes the formation of putrescine. This rate-limiting step may be blocked by various substrate analogs.[2,3] In our investigations we have used DL-α-difluoromethylornithine, an enzyme-activated irreversible inhibitor of ODC.[3,4]

In order to determine whether the polyamines are required also for the early development of vertebrates, we have analyzed the effects of DL-α-difluoromethylornithine on chick embryogenesis. In the chick embryo there is an early biphasic activation of the ODC activity, with peak activities at 15 and 23 h of development, coinciding with gastrulation and neurulation, respectively.[5] All three polyamines varied in a similar manner as did ODC.[5] DL-α-Difluoromethylornithine treatment markedly interfered with the synthesis and accumulation of polyamines that begin already during cleavage (Table 1). The ODC activity decreased significantly during the 16 h treatment period, permitting only a slight increase in polyamine concentrations. The fact that there was no complete inhibition of the ODC activity is probably due to the high turnover rate of this enzyme. The morphological consequences of polyamine depletion were appalling; there was no development beyond the primitive streak stage (gastrulation), *i.e.* no neurulation or formation of somites.[5] These results are in line with what has been found in invertebrate embryos,[1] and corroborate our theory that pregastrular stimulation of polyamine synthesis is a ubiquitous event associated with the activation of the embryo genome occurring at gastrulation.

Experimental. Fertilized White Leghorn eggs were incubated at 37.5 ± 0.5 °C. Pooled embryos, 5 from each stage, were sonicated at 4 °C in 200 μl of 10 mM Tris/HCl (pH 7.2) containing 0.5 mM Na_2EDTA, 5.0 mM dithiothreitol and 0.05 mM pyridoxal 5'-phosphate. A 100 μl aliquot was analyzed for ODC activity essentially as described by Jänne and Williams-Ashman.[6] The release of $^{14}CO_2$ during the ODC-catalyzed formation of putrescine from DL-1-^{14}C-ornithine monohydrochloride (18.5 MBq/mmol) was measured. For polyamine analysis pooled embryos were sonicated in 0.2 M perchloric acid at 4 °C, and the extracts were analyzed by a thin-layer chromatographic method as described by Seiler.[7]

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Table 1. Effects of DL-α-difluoromethylornithine on polyamine metabolism in the early chick embryo.

Treatment[a]	Developmental age (h) at		ODC-activity/%	Concentration of		
	Addition	Assay		Putrescine/%	Spermidine/%	Spermine/%
Control	5	5	100[b]	100[c]	100[c]	100[c]
	5	12	637	221	220	215
	5	18	484	336	490	380
	5	21	605	397	570	400
DL-α-Difluoromethylornithine	5	12	33	124	130	180
	5	18	24	155	195	175
	5	21	36	154	172	159

[a] At 5 h of development 100 μl of 0.93 % NaCl (control) or 42 μmol of DL-α-difluoromethylornithine in 100 μl of 0.93 % NaCl were injected directly beneath the blastoderm. [b] 100 % corresponds to 0.59 nmol of $^{14}CO_2$ h^{-1} (mg protein)$^{-1}$. [c] 100 % corresponds to 0.82, 0.55, and 0.50 nmol (mg protein)$^{-1}$ of putrescine, spermidine and spermine, respectively.

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