Histological localization by fluorescent hormone conjugate has been used to determine the histological localization of hormones in various nonneuronal tissue (*10*). In these earlier studies, however, competitive inhibition by excess unlabeled hormone was not included. Bioactive rhodamine-conjugated enkephalin was recently used in neuroblastoma cells to study the distribution of binding sites (*11*). The use of bioactive fluorescent derivatives of peptides in brain tissue can become a fast and accurate method for demonstrating the distribution of receptor regions in brain tissue.

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Role of Ornithine Decarboxylase in Cardiac Growth and Hypertrophy

Abstract. *Inhibition of cardiac ornithine decarboxylase (ODC) by α-difluoromethylornithine (DFMO) did not prevent normal cardiac growth in mature rats but attenuated isoproterenol-induced hypertrophy. Hypertrophy caused by triiodothyronine was not prevented by DFMO. There appear to be both ODC-dependent and ODC-independent processes contributing to the subcellular mechanisms associated with growth, which must be considered in the potential laboratory and clinical use of DFMO.*

Ornithine decarboxylase (ODC) (E.C. 4.1.1.17) catalyzes the first and probably rate-limiting step in the biosynthesis of the polyamines putrescine, spermidine, and spermine (*1*, *2*). Polyamines are thought to play regulatory roles in both nucleic acid and protein syntheses, and the ODC molecule itself may act as an initiating factor for RNA polymerase I (*3*). Studies with regenerating rat liver, tumor cells, and a variety of prokaryotic and eukaryotic systems have demonstrated that ODC activity and polyamine concentrations are highest during rapid growth, differentiation, or replication and decrease as these processes cease or as the number of growing or dividing cells declines (*3*, *4*). Although these observations imply a functional role for ODC and the polyamines in the regulation of cellular development, not until recently has direct proof of this hypothesis been obtainable; the development of α-difluoromethylornithine (DFMO) (RMI 71, 782), a potent, selective, enzyme-activated irreversible inhibitor of ODC (*5*), has made it possible to show that ODC activity is necessary if cell replication is to progress normally. Incubation with DFMO retards the multiplication of cells in culture, and the growth of tumors in vivo and in vitro is inhibited by the drug (*6*). During mammalian development, DFMO causes the arrest of embryogenesis (*7*).

It is unclear, however, whether ODC is obligatory in all growth processes or whether only replication is affected. One way of testing this hypothesis is to examine the effects of inhibition of ODC in tissues that grow by hypertrophy of preexisting cells rather than by replication, a circumstance that prevails in the mature rat heart (*8*). Cardiac hypertrophy can be produced by aortic constriction, sympathetic hyperactivity, hyperthyroidism, or chronic stress or exercise (*9*), and in each case the changes in heart weight are preceded by ODC stimulation. In our study, DFMO has been used to determine if ODC activity is necessary for the normal growth of the mature heart and whether stimulation of ODC is an obligatory step in sympathetically induced (with isoproterenol) or hormonally induced (with triiodothyronine) cardiac hypertrophy.

Male Sprague-Dawley rats (Zivic-Miller) with initial body weights of 160 to 180 g were housed two per cage and given unlimited amounts of food and water. Cardiac hypertrophy was produced by daily subcutaneous administration of *dl*-isoproterenol (ISO) (0.5 mg/kg) or 3,3′,5-triiodo-*l*-thyronine (T_3) (0.1 mg/kg) (both from Sigma Chemical Company). Rats were killed by decapitation, and the heart ODC activity was determined in the 26,000*g* supernatant of hypotonic tris homogenates by a modification of the method of Russell and Snyder (*2*, *10*); heart and body weights were recorded, and the ratio of heart weight to body weight (a more specific index of cardiac hypertrophy) was calculated. Results are reported as the means ± the standard errors of the means of six or more animals, with levels of significance calculated by the two-tailed *t*-test.

Subcutaneous administration of 200 mg/kg of DFMO resulted in a rapid and profound decline in cardiac ODC, and

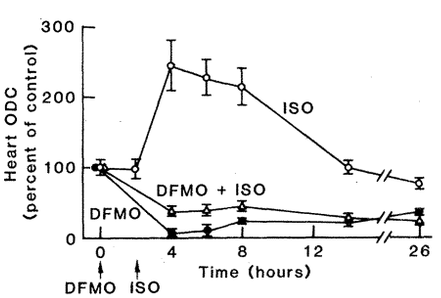

Fig. 1. Time course of cardiac ODC activity after a single treatment with DFMO or ISO, or both. Control ODC activity was 1.29 ± 0.14 nmole hour^{-1} g^{-1}.

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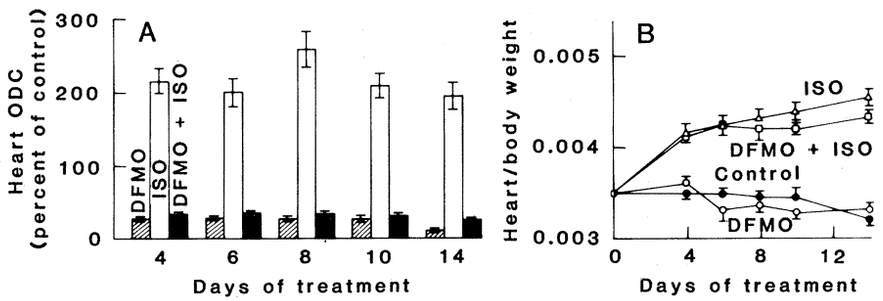

Fig. 2. Heart ODC (A) and the ratio of heart weight to body weight (B) during repeated daily treatments with DFMO or ISO, or both. In each case, animals were initially given saline or DFMO and then saline or ISO 2 hours later. The animals were killed 4 hours after the second injection.

enzyme levels were still very low after 26 hours (Fig. 1). Similarly, DFMO completely prevented the elevation of cardiac ODC caused by ISO. With daily administration of the drugs for 14 days, identical ODC stimulation by ISO alone and identical inhibition by DFMO of both basal ODC and of the ISO effect were obtained on each day (Fig. 2A). Despite the clear-cut effectiveness of DFMO, this drug failed to interfere with normal cardiac growth, since control heart weights increased from 471 ± 13 mg at the beginning of the experiment to 900 ± 25 mg 14 days later, as did heart weights in the DFMO group (500 ± 17 mg to 946 ± 31 mg). Moreover, DFMO did not prevent the initial phase of ISO-induced cardiac hypertrophy (Fig. 2B), but a small attenuation of the hypertrophy became noticeable beginning after 8 days of treatment. This finding suggested that DFMO might be effective if treatment with DFMO were begun well before ISO administration was initiated. To test this possibility, we administered DFMO to rats daily for 10 days prior to the start of daily ISO administration; the rats were killed 4 days after ISO administration was started. Inhibition of ODC was apparent in both DFMO and DFMO + ISO groups (Fig. 3A). In this case, however, the cardiac hypertrophy was significantly attenuated by as much as 50 percent (Fig. 3B). This effect was not due to interference by DFMO with the physiological effect of ISO; full cardioacceleration was obtained even after 14 days of DFMO treatment, as assessed by heart rate measurements in urethane-anesthetized rats (increase after ISO = 183 ± 12 beats per minute in controls, 187 ± 7 beats per minute in DFMO-treated rats). Neither DFMO alone nor DFMO in combination with ISO produced significant changes in body weight.

In contrast to the inhibition by DFMO of ISO-induced hypertrophy, even a 10-day pretreatment with DFMO did not block hypertrophy caused by T_3 (Fig. 3B), despite complete effectiveness in preventing the rise in ODC (Fig. 3A). This result indicates that there are fundamental differences in the action of ODC in cellular mechanisms by which thyroid hormones and sympathomimetics elicit increased cardiac growth.

The results in the heart can be contrasted with the relatively complete effectiveness of growth inhibition by DFMO in a variety of systems in which cell replication is occurring (*6*). It is apparent that ODC activity is not essential for normal growth of the adult heart and that the enzyme participates only partially in ISO-induced hypertrophy; therefore, in the latter case, at least two subcellular mechanisms appear to be involved, one ODC-sensitive and one ODC-insensitive. For T_3 only an ODC-insensitive mechanism operates. The use of DFMO thus provides a method for the identification and characterization of some of the events linking activation of target organ receptors to myocardial growth. The potential clinical use of DFMO to prevent various forms of cardiac hypertrophy would appear to depend upon the type of signal initiating the growth process; the drug would appear to be innocuous toward normal growth and would be ineffective in dealing with hypertrophy secondary to hyperthyroidism, but it could be of use in hypertrophy caused by sympathetic hyperactivity or pheochromocytoma.

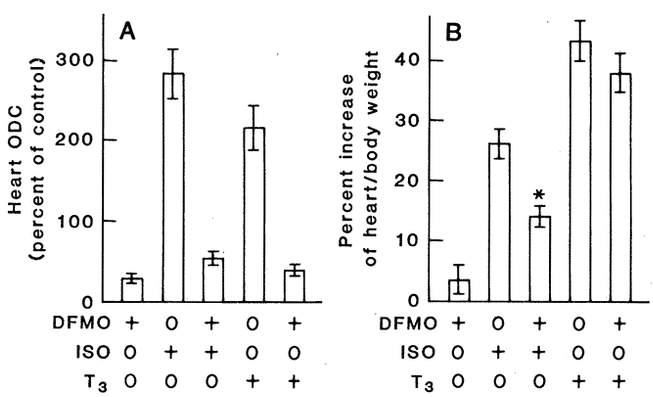

Fig. 3. Heart ODC (A) and the ratio of heart weight to body weight (B) in rats pretreated for 10 days with DFMO or saline. From day 11 to day 14, animals received DFMO or saline and then saline, ISO, or T_3 2 hours later. Rats were killed 4 hours after ISO administration and 24 hours after T_3 administration, since these are the times at which ODC stimulation is maximal for each agent (*9*). The asterisk denotes that the weight ratio of the DFMO + ISO group is significantly lower than that of the group given ISO alone ($P < .002$) but higher than that of the group given DFMO alone ($P < .001$).

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