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ISOPROTERENOL-INDUCED RESPONSES IN THE REGIONAL POLYAMINE METABOLISM OF THE RAT HEART

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SUMMARY. Rats were treated with a single dose of isoproterenol (25 mg/kg s.c.) and the levels of polyamines determined in various parts (right ventricle, basis, medial part and apex of the left ventricle) of the heart 24, 48 and 72 hours after the injection. The isoproterenol treatment produced marked alterations in the concentrations of cardiac polyamines. The most apparent changes were seen in the apex and medial part of the left ventricle where spermidine concentration exhibited a biphasic response with peaks at 24 and 72 hours. In the basis of the heart the spermidine concentration was significantly elevated only at 24 hours. In the right ventricle the spermidine level was significantly higher than control at 72 hours. Spermidine/spermine ratio was augmented in all cardiac tissues examined over the 72-hour period. Results appear to show that the isoproterenol-induced alterations in cardiac polyamine metabolism were not uniformly distributed in the various regions of the heart.

INTRODUCTION

Several experimental evidences suggest that the natural polyamines (putrescine, spermidine and spermine) are involved in cell growth, hypertrophy and division (1-4). They are intimately correlated with protein and nucleic acid synthesis (5-7). Cardiac polyamine levels and the activity of ornithine decarboxylase (L-ornithine carboxy-lyase, EC 4.1.1.17) the initial and rate limiting enzyme in the polyamine biosynthetic pathway have been shown to be enhanced in various forms of experimentally induced cardiac hypertrophy (8-14). Rat heart

ABBREVIATIONS: ODC, ornithine decarboxylase; DFMO, α-difluoromethylornithine; T_3, 3,3',5-triiodo-l-thyronine; PDE, phosphodiesterase.

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ornithine decarboxylase (ODC) activity has been demonstrated to increase after a single low dose of isoproterenol in hours (15-19) and a new high-affinity form appears (20).

Inhibition of cardiac ODC by α-difluoromethylornithine (DFMO) a potent, selective, enzyme-activated irreversible inhibitor of ODC, attenuated the isoproterenol-induced cardiac hypertrophy in rats but neither normal growth nor hypertrophy caused by 3,3',5-triiodo-l-thyronine (T_3) was prevented by DFMO (21). This finding suggests that there are fundamental differences in the action of ODC in cellular mechanisms by which thyroid hormones and sympathomimetics elicit increased cardiac growth.

Earlier data have shown that the isoproterenol-induced alterations are not uniformly distributed in the heart, they are the most prominent in the apical tissue and are diminished towards the basal part of the left ventricle as has been shown by the sensitive mercurascan and [^{3}H]tetracycline uptake techniques (22, 23). Therefore the present investigations were designed to study the changes in cardiac polyamine concentrations over a 72-hour-period after a single dose of isoproterenol to ascertain whether the alterations in the polyamine metabolism produced by isoproterenol in those areas are uniform or not.

MATERIALS AND METHODS

Male albino rats from the CFY (LATI, Hungary) inbred strain weighing 180-260 g were used. The experimental group received a single dose (25 mg/kg s.c.) of isoproterenol. Controls were injected with physiologic saline of the same volume. At 24, 48 and 72 hr after isoproterenol treatment the animals were exsanguinated under ether anaesthesia at the same period of day (between 9 and 10 a.m.). Controls were sacrificed at 24 hr. The hearts were rapidly excised,the atria separated from the right ventricle (17.8-18.9 % of heart weight). The left ventricle was subsequently sectioned into three parts: basal (37.7-41.3 %), medial (30-33 %) and apical (9.5-13.2 %) parts as described by Faltová et al. (22). All material was kept on iced glass vessels. The respective parts of two rat hearts were pooled and

weighed. The iced tissue was homogenized in 2.5 ml (for the apical tissue) or 3.5 ml (for other parts of the heart) cold PCA (2 % w/v) using an Ultra-Turrax homogenizer. The homogenate was centrifuged (10.000 x g, 30 min, 5°C) and the supernatant fluid was processed for polyamine determination (24) except that after the ion-exchange chromatography the samples evaporated were redissolved in 0.5 ml $NaHCO_3$ (1 % w/v) and centrifuged for 5 min. Aliquots of the supernatant were dansylated according to the method of Seiler (25). The dansyl polyamines were subjected to thin layer chromatography on pre-coated plastic silica-gel sheets (Art 5748 E. Merck, Darmstadt) using ethylacetate-cyclohexane(2:3) solvent mixture. The fluorescent spots were identified under u.v. light using polyamine reference standards run on the same sheet. The spots were cut out and extracted in 3.0 ml of methanol + conc. ammonia (95:5) solution. Fluorescence measurements were done in a Locarte LFM/5 fluorescent spectrum fluorimeter equipped with a mercury arc lamp using an LF 2 primary filter (transmission from 340-350 nm), and a wedge monochromator set at 505 nm on the secondary side.

Chemicals used: spermidine 3.HCl (Serva), spermine 4.HCl (Serva), putrescine (tetramethylenediamine, Koch-Light Laboratories Ltd), dansyl-chloride (5-dimethylamino-1-naphthalene sulfonylchloride, Serva). Isoproterenol (Propylon EGYT Pharmacochemical Works, Budapest, Hungary). Statistical significance was calculated by Student's t test. Results are expressed as means $\pm$ S.E.

RESULTS AND DISCUSSION

The concentration of polyamine in different parts of the rat heart is shown in Fig. 1. Isoproterenol treatment resulted in substantial alterations of cardiac polyamine metabolism. In the basal part of the left ventricle spermidine concentration was significantly increased (126.2 % of control) at 24 hr but was at about control level at 48 and 72 hr. Spermine concentration was significantly reduced at 24 (70.6 %) and 48 hr (61.4 %) and restored to about the initial level by 72 hr. Putrescine concentration was not changed significantly over the 72-hr observation period. In the medial part the changes of the spermidine content exhibited a biphasic response with two peaks, the first occurred at 24 hr (196.6 %), the second one at 72 hr (151.3 %). In the apical tissue putrescine concentration was significantly augmented at 72 hr (263.4 %). The pattern of the spermidine response was similar to that observed in the medial part except that the 72 hr surge (210.3 %) was even more

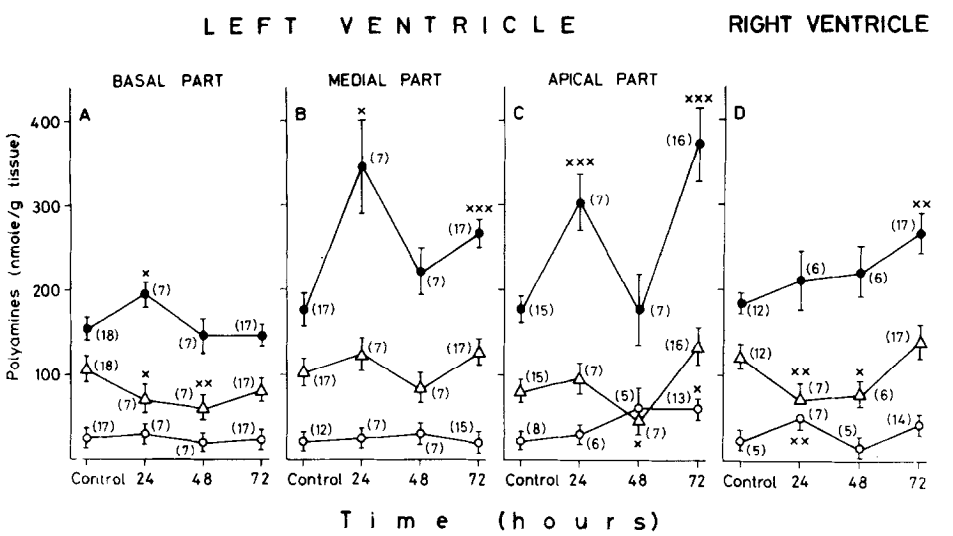

Fig. 1. Effect of isoproterenol treatment on the polyamine concentrations in the basal (A), medial (B) and apical (C) parts of the left ventricle and in the right ventricle (D) of the rat heart. Rats were injected subcutaneously with a single dose (25 mg/kg) of isoproterenol and killed at times indicated. The respective parts of two hearts were pooled and processed for polyamine determination as described in Materials and Methods. Putrescine (o——o), spermidine (●——●), spermine (Δ——Δ). Values are means ± S.E. Number of observations are in parentheses. Level of statistical significance (Student's t test): x $p < 0.05$, xx $p < 0.01$, xxx $p < 0.001$ in comparison with control.

apparent. The spermine content was significantly depressed at 48 hr (67.3 %). In the right ventricle the putrescine concentration was significantly elevated (218.4 %) only at 24 hr. The spermidine content (144.9 %) increased significantly at 72 hr, whereas the concentration of spermine was significantly reduced at 24 (58.4 %) and 48 hr (65.9 %). The spermidine/spermine ratio was elevated in all cardiac tissues examined over the 72-hour observation period (data not shown).

The dose of isoproterenol applied in the present study produces cardiac hypertrophy (26). Isoproterenol treatment results in an elevation of tissue polyamine levels in mouse salivary glands (27, 28). Other data, however, indicate that the spermidine concentration in the mouse parotid glands decrease significantly during the first 6 hr after a single

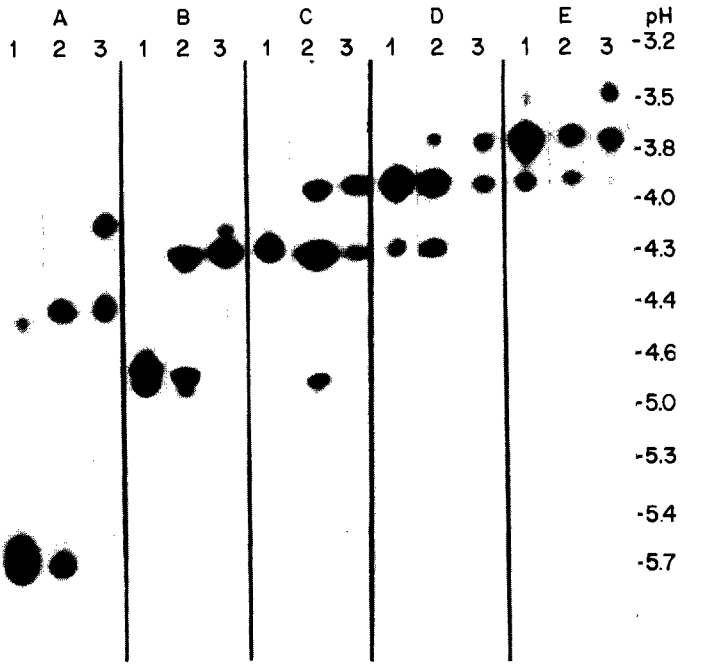

Figure 2. Isoelectric focusing autoradiograph of ^{125}I-EGF species after internalization.

Two groups of culture dishes of Rat-1 cells (1 x 10^6/35mm dish) were incubated at 37°C in 0.5 ml DMEM media containing 1 mg/ml serum albumin and 4 x 10^6 CPM of each of the ^{125}I-EGF species shown in lanes 1-5 of Figure 1. After 15 min one group of cells was removed for extraction of both surface-bound and internalized ^{125}I-EGF (see Table II for extraction procedure). At that time 5 µg non-radioactive EGF was added to the remaining culture dishes for 60 min to serve as a cold chase. These cells were extracted similarly to the first group. Internalized ^{125}I-containing cell extracts were dialyzed against H_2O, concentrated, and applied to isoelectric focusing gels. Dried gels were exposed for autoradiography. ^{125}I-EGF species employed for the experiment had isoelectric points of 5.6 (panel A), 4.5 (panel B), 4.3 (panel C), 4.1 (panel D) and 3.8 (panel E). Lane 1 of each panel represents the ^{125}I-EGF species prior to cell binding. Lane 2 represents intracellular ^{125}I activity 15 min after cell binding, and lane 3 represents intracellular ^{125}I activity after the 60 min cold EGF chase.

purified forms of ^{125}I-EGF and were examined by isoelectric focusing. Cells were incubated at 37°C for 15 min, at which time the surface-bound and internalized ^{125}I-compounds were extracted. An identical set of cells were incubated with unlabelled EGF for 60 min after the 15 min labelling period and then similarly processed by sequential removal of external and internal ^{125}I-compounds. The internalized compounds were examined by isoelectric focusing.

Each of the ^{125}I-EGF forms which was bound was processed to more acidic forms during the course of the experiment (Figure 2). By the end of the 15 min pulse-labelling period, substantial amounts of the ^{125}I-EGF species shown

Results of this study appear to demonstrate that a single dose of isoproterenol elicited marked alterations in cardiac polyamine metabolism. The responses observed in various parts of the heart were not, however, quite uniform. The medial part and apical tissue of the left ventricle exhibited the most apparent responses. The basis was less responsive, whereas the right ventricle displayed a response pattern dissimilar to those noted in the other parts of the heart. The elevated spermidine/spermine ratio observed in all cardiac regions after isoproterenol injection is a condition that favors cell division and regeneration in other tissues (35, 36).

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