Cardiac responses to pituitary and thyroid hormones

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Both growth and thyroid hormones may be essential for the development of certain forms of cardiac hypertrophy (Fanburg, 1970). Organ growth accompanied by increased metabolic demand results in increased activities of glycolytic enzymes (Roberts & Szego, 1953). Since enhanced enzyme responses can be a consequence of hormonal action (Weber, Singhal, Stamm, Lea & Fisher, 1966), the effects of daily treatment of hypophysectomized (HPX) and hypothyroid (HYPOT) rats with growth hormone (GH) and thyroid hormones (T3 & T4) on myocardial hexokinase (HK), phosphofructokinase (PFK) and pyruvate kinase (PK) as well as on glycolytic intermediates were studied.

Four weeks after hypophysectomy or induction of the hypothyroid state, decreases in all three enzyme activities occurred. Treatment with growth hormone enhanced myocardial hexokinase and pyruvate kinase, but thyroid hormone only myocardial hexokinase and pyruvate kinase. In hypothyroid rats, all thyroid hormone treatments increased myocardial hexokinase and pyruvate kinase; phosphofructokinase enhancement resulted only after administration of 4 μg thyroid hormone for one week.

Fluorimetric measurement of eleven glycolytic intermediates after freezing hearts *in vivo* at 5 s intervals up to 25 s subsequent to cutting the blood supply, demonstrated increased phosphofructokinase, glyceraldehyde 3-phosphate dehydrogenase and pyruvate kinase catalysed reaction products at 20 seconds. These activations did not occur in hypophysectomized rat hearts. Treatment of HPX rats for 1 and 7 days with 70 μg thyroid hormones and 0·5 mg growth hormone/day resulted in an

TABLE 1. *Effect of thyroid and growth hormone treatment of hypophysectomized and hypothyroid rats on the activities of myocardial enzymes*

Enzyme activities ((μM substrate metabolized/h)/g wet tissue)±S.E.

Treatment	Hexokinase	Phosphofructokinase	Pyruvate kinase
Sham HPX	240± 7	756±23	7890±220
HPX	209± 3ø	452±29ø	5280±190ø
HPX+GH (2 wk)	219± 9	1103±29*	6480±300*
HPX+GH (4 wk)	238±14*	1020±13*	6060±100*
HPX+T4 (2 wk)	235± 4*	549±100	6390±160*
HPX+T4 (4 wk)	241±11*	284±48	6720±140*
Control	261± 9	2100±20	8920±230
Hypot	174± 1ø	1750±90ø	6580±220ø
+T3 (4·0 μg 1 day)	220±17*	1830±10	8400±160*
+T3 (0·4 μg 1 wk)	257±19*	1670±80	7140±230
+T3 (2·0 μg 1 wk)	260±12*	1700±60	10230±300*
+T3 (4·0 μg 1 wk)	263± 1*	2130±90*	9420±180*

Each value represents the mean ± S.E. of enzyme activity in homogenates prepared from three groups of cardiac tissue, each pooled from three animals. HPX, 4 weeks after surgical hypophysectomy; HYPOT, hypothyroid 4 weeks after intraperitoneal injection with 800 μCi carrier-free ^{131}I/100g; +GH, 1·0 mg growth hormone/day; +T4, 50 μg thyroxine/day; +T3, designated dose of tri-iodothyronine/day—all administered subcutaneously/100 g body weight. Significant decreases *c.v.* sham HPX and control denoted by ø; increases *c.v.* HPX and HYPOT denoted by * ($P<0·05$).

increased activity in glycolytic pattern after 20 s ischaemia, as was observed in normal hearts.

Relative to earlier findings in fluoracetate treated hypothyroid rats (Paterson, 1971) these results indicate that phosphofructokinase responds to growth hormone rather than to thyroid hormones with the treatments used, but hexokinase and pyruvate kinase respond to either. It may therefore be concluded that both these hormones are necessary for maintaining a normal cardiac response to ischaemia of short duration as well as to long-term hypertrophy induced in the rat heart (Beznak, 1967).

REFERENCES

BEZNAK, M. (1967). The role of growth hormone and thyroxine in nephrogenic hypertension and cardiac hypertrophy in hypophysectomised rats. *Can. J. Physiol. Pharmac.*, **45**, 993–1000.

FANBURG, B. L. (1970). Experimental cardiac hypertrophy. *New Eng. J. Med.*, **282**, 723–732.

PATERSON, R. A. (1971). Metabolic control of rat heart glycolysis after acute ischaemia. *J. mol. Cell. Cardiol.*, **2**, 193–210.

ROBERTS, S. & SZEGO, C. M. (1953). Steroid interaction in the metabolism of reproductive targen organs. *Physiol. Rev.*, **33**, 593–629.

WEBER, G., SINGHAL, R. L., STAMM, N. B., LEA, M. A. & FISHER, E. A. (1966). Synchronous behaviour pattern of key glycolytic enzymes. *Adv. Enzyme Reg.*, **4**, 59–81.

Mitogenic and inflammatory activities produced by antigen activation of guinea-pig lymphocytes

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Lymphocytes can be stimulated *in vitro* by several agents to release materials into the culture medium with a wide variety of biological actions (Bloom & Glade, 1971). After specific antigen activation of cells from animals (or man) with delayed hypersensitivity, a class of materials is generated which has been proposed to mediate cellular immunity and termed lymphokines (Dumonde, Wolstencroft, Panayi, Matthews, Morley & Howson, 1969). Two of the biological activities observed in these preparations are (a) the appearance of inflammation after intradermal injection into normal animals and (b) the ability to increase DNA synthesis of cultured lymphocytes. In the present experiments, an attempt has been made to correlate the inflammatory activity of these preparations with their mitogenic activity. Inflammatory activity was assessed by the measurement of accumulation of ^{125}I-labelled serum albumin after injection into guinea-pig skin over a period of 4 hours. Mitogenic activity was assessed by measurement of the increased incorporation of tritiated thymidine, induced in cultured guinea-pig lymph node lymphocytes.

Guinea-pigs were sensitized with antigen (100 μg of bovine gamma-globulin in Freund's complete adjuvant). Lymphocytes collected 2 weeks after sensitization were cultured in serum-free Eagle's medium for 18–24 h in the presence of antigen (1 mg/ml). Cells were separated by centrifugation and the culture fluid stored at −20° C.

In such preparations, biological activities were greatest in preparations obtained by stimulating cells with specific antigen when compared with preparations obtained from cells cultured either in the absence of antigen or with a non-cross reacting antigen (for example ovalbumin). Both inflammatory and mitogenic activities of such preparations were greater than those due to extracts from equivalent numbers of cells