Turnover Rates of Myocardial Proteins in the Rat

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In studying the role of insulin in the regulation of protein synthesis in the heart, it became of interest to measure the rate of renewal of heart protein. The technique of constant infusion of labelled amino acids may be used to obtain synthetic rates of mixed tissue proteins *in vivo* (Waterlow & Stephen, 1968). The isolated-organ perfusion situation allows simplification of the theoretical analysis used for the constant-infusion experiments. Values for the half-lives of synthesis of myocardial protein fractions have been obtained in isolated rat hearts perfused with L-[U-¹⁴C]tyrosine at constant specific radioactivity. Perfusions were performed retrogradely via the aorta (Langendorff technique), in the presence and in the absence of insulin, and also via the left atrium, these hearts performing external mechanical work (Chain *et al.*, 1969). Measurements of tyrosine specific radioactivity in perfusate, in intracellular fluid and in hydrolysed heart protein were made by enzymic decarboxylation and fluorimetry (Garlick & Marshall, 1972).

The specific radioactivity of intracellular free tyrosine rapidly rose to a plateau value in the perfused heart, and it remained constant for the duration of an hour's perfusion. There was no decline in perfusate specific radioactivity over this time. In Langendorff hearts perfused either with or without insulin the specific radioactivity of the free intracellular tyrosine reached a value of about 70% that in the perfusate; in working hearts intra- and extra-cellular specific radioactivities became nearly equal.

In working hearts, and in Langendorff hearts perfused with insulin, $19 \pm 2\%$ of total heart protein is turned over/day (corresponding to a half-life of 3.7 ± 0.3 days). The turnover rates of myofibrillar and sarcoplasmic proteins did not differ significantly.

The heart contains a large number of different proteins, which might show a wide range of synthetic rates. To obtain an indication of whether there exists a contractile protein with a particularly rapid turnover, which might selectively respond to hormonal stimulation, myofibrillar proteins prepared from hearts perfused under different conditions with ¹⁴C-labelled amino acids were separated by polyacrylamide-gel electrophoresis (Sender, 1971). An estimate of the specific radioactivity of individual proteins was made by gel densitometry and by slicing of the gels for liquid-scintillation counting. No protein could be detected with a particularly high specific radioactivity.

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Insulin caused an overall increase in specific radioactivity, and did not preferentially increase the labelling of any singly myofibrillar protein.

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Insulin and Protein Synthesis in the Perfused Rat Heart

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Insulin has been shown to regulate the metabolism of glucose in the rat heart by a specific stimulation of reactions on the pathway to glycogen synthesis (Chain *et al.*, 1969). The hormone has also a stimulatory effect on the incorporation of ¹⁴C-labelled amino acids into heart protein. It was decided to investigate whether the insulin stimulation of ¹⁴C-labelled amino acid incorporation depended on intracellular glucose metabolism by using an isolated perfused rat heart preparation.

Hearts were pre-perfused with a medium lacking any oxidizable substrate to deplete the content of endogenous glucose, glucose 6-phosphate and glycogen. They were then perfused with 10mM-pyruvate and ¹⁴C-labelled amino acids for a further period by recirculation. Insulin was found to cause an increase in specific radioactivity of proteins in these hearts of a magnitude (approx. 50%) similar to that found in control hearts perfused with glucose (5mM) and not subjected to the period of substrate-free perfusion.

When hearts were perfused with Krebs-Henseleit buffer containing 5mM-2-deoxy-D-glucose, an inhibitor of glucose utilization (Kipnis & Cori, 1959), they soon showed signs of failure, developing arrhythmias and a rapidly falling cardiac output. Cardiac arrest occurred in 5–10min. The addition of pyruvate (10mM) to the 2-deoxyglucose-poisoned heart before arrest rapidly reversed the failure, and the general condition of the heart, as assessed by the physiological criteria used, returned to normal after 2–3min. 2-Deoxyglucose did not affect protein synthesis. Insulin caused a 50% increase in ¹⁴C-labelled amino acid incorporation into protein of hearts perfused with pyruvate and 2-deoxyglucose. It was thus

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