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UPTAKE AND RELEASE OF IMMUNOREACTIVE INSULIN IN CORONARY CIRCULATION IN MAN: STUDIES AT REST, DURING EXERCISE, AND DURING GLUCOSE AND INSULIN INFUSIONS

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SUMMARY

Signficant uptake and release of immunoreactive insulin by the heart have been observed in man, and this is related to plasma insulin levels. Exercise and the fed state appear to affect the myocardial handling of insulin. The findings could not be related to myocardial carbohydrate metabolism, but could, during exercise, be related to myocardial lipid metabolism.

INTRODUCTION

When insulin exerts its action on the heart, it is presumably taken up from arterial blood by the myocardium, where it interacts with receptors. Recent work has clarified the nature and control of receptors for insulin (Forgue and Freychet, 1975; Freychet, 1975; Raff, 1976). There remains, however, little information with regard to the peripheral handling of insulin *in vivo* and how this relates to arterial insulin concentrations (Asmal *et al.*, 1971). We have examined the coronary arteriovenous difference in insulin immunoreactivity in man from this point of view and have also assessed its relationship to myocardial substrate utilization.

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METHODS

Subjects were male volunteers either healthy or with suspected ischemic heart disease. They were examined after an overnight fast. Simultaneous brachial artery and coronary sinus blood samples were obtained (Wahlqvist *et al.*, 1972; Carlson *et al.*, 1973, 1975). Heparin was not administered. Some subjects were examined during prolonged exercise in the supine position on a cycle ergometer (Kaijser *et al.*, 1972) following observations at rest. Four situations were considered: 1) fasting, 2) insulin infusion to raise arterial insulin concentrations, 3) nicotinic acid infusion where arterial free fatty acid (FFA) concentrations were kept low and arterial insulin concentrations tend to be low (Lassers *et al.*, 1972), and 4) a simulated fed state where glucose and a fat emulsion, Intralipid®-S, were infused and arterial insulin concentrations elevated (Carlson *et al.*, 1973, 1975; Wahlqvist *et al.*, 1974).

In most categories, subjects were given oral iodine (Lugol's solution) followed by an intravenous injection of about 6μCi of ^{125}I-albumin as a tracer for plasma albumin two days before the investigation. This enabled estimation of any change in plasma protein concentration as an index of shift of plasma water across the coronary circulation.

Plasma samples for insulin determination were frozen at -20° C until assayed. Insulin was determined using either a radioimmunoassay kit (Radiochemical Centre, Amersham, England) (Hales and Randle, 1963) or a radioimmunoabsorbent technique (Pharmacia AB, Uppsala) (Wide, Axén, and Porath, 1967). Where insulin was infused, ten replicates of arterial and of coronary sinus plasma were assayed so that the significance of each observation of an arteriovenous difference could be assessed. In the series in which an intravenous fed state was created, each arterial and coronary sinus sample was assessed in three dilutions, each of these in triplicate. In all cases the sample curve was parallel to that of the standard. Other assays were made in duplicate.

Blood samples for determination of glucose, lactate, pyruvate, FFA, triglyceride, "exogenous triglyceride" (plasma triglyceride circulating as Intralipid emulsion), and ^{125}I-albumin were collected as previously described (Kaijser *et al.*, 1972; Lassers *et al.*, 1972; Wahlqvist *et al.*, 1972; Carlson *et al.*, 1973, 1975).

RESULTS

Uptake

When insulin was infused intravenously in two subjects, each subject had a significant arterial-coronary sinus difference in immunoreactivity (Ia-cs) (Table 1). Ia-cs was also significant and further increased when glucose influsion was combined with insulin infusion.

Table 1. Significance of single observations of extraction of immunoreactive insulin from coronary blood

Subject§	Fasting		Insulin infusion		Insulin and glucose infusion	
	Ia‡	Ia-cs‡	Ia‡	Ia-cs‡	Ia‡	Ia-cs‡
FR	3.9 ±0.3	−1.3 ±0.6^{ns}	28.7 ±0.4	2.4 ±0.6**	154.3 ±5.6	37.6 ±9.0***
GR	12.1 ±0.3	0.6 ±0.4^{ns}	35.9 ±0.9	4.4 ±1.1**		

†Ia refers to insulin immunoreactivity in arterial blood and Ia-cs to the arterial-coronary sinus difference in insulin immunoreactivity. Significance of Ia-cs is indicated by ns ($p > 0.05$), **($p < 0.01$), ***($p < 0.001$).

§Subject FR had a doubtful anginal syndrome and was given an infusion of 2U actrapid insulin in 1% albumin and 0.9% saline per hour. This infusion was then combined with that of 0.4g 20% glucose/min. Subject GR had angina pectoris and the rate of insulin infusion was 4U/hr. Each infusion lasted 1 hr.

‡Means ±S.E.M. are shown.

In the intravenous fed state, where infusions of glucose and a fat emulsion were given, resting subjects did not as a group demonstrate a significant myocardial insulin extraction (Ia-cs) (Table 2). When parenterally fed subjects exercised, however, a significant Ia-cs was observed.

Release

There were two circumstances in which a significant release of immunoreactive insulin into coronary venous plasma was observed. One was where fasting subjects exercised for a prolonged period (Table 2). This was associated with a lower arterial insulin immunoreactivity than at rest. This is in contrast to the myocardial handling of insulin during comparable exercise when infusions of glucose and a fat emulsion were given. In that circumstance insulin was taken up.

Insulin release was observed as well during the infusion of nicotinic acid (Table 2). During nicotinate infusion, plasma FFA concentrations were 250 ± 20 μmol/liter, compared with control observations when they were 650 ± 50 μmol/ liter, reflecting the antilipolytic action of nicotinic acid. Although arterial insulin immunoreactivities tended to be lower when nicotinic acid was infused, they were not significantly so.

Water Shifts

Possible water shifts leading to alterations in insulin immunoreactivity across the coronary circulation were assessed by observing the arterial-coronary sinus difference in ^{125}I-albumin. These changes ranged from −1.0 to 0.1% of the arterial levels. The −1.0% change was during the infusion of nicotinic acid when

Table 2. Arterial plasma insulin† immunoreactivity (Ia) and arterial-coronary sinus difference in plasma insulin immunoreactivity (Ia-cs)§ in different experimental categories‡

	Fasting		Fasting and nicotinic acid††		I.V. fed state§§	
	Rest (12)	Exercise‡‡ (12)	Rest (9)	Exercise‡‡ (9)	Rest (36)	Exercise‡‡ (8)
Ia	17.9 ±0.9	13.8 ±0.8	15.2 ±1.4	11.4 ±1.4	26.8 ±3.2	10.4 ±2.9
Ia-cs	−1.1 ±0.8^{ns}	−2.8 ±0.9**	−2.3 ±0.6**	−1.9 ±1.2^{ns}	1.2 ±1.0^{ns}	3.3 ±1.0*

†Units are μU/ml.

§Significance of Ia-cs is indicated by ns ($p > 0.05$), * ($p < 0.05$), ** ($p < 0.01$).

‡Number of observations for each category is shown in parentheses. In each category, mean ±S.E.M. is shown.

†Nicotinic acid was infused intravenously at a rate of 200 mg/hr or 400 mg/hr.

§§"I.V. Fed State" refers to the infusion of 0.4 g 20% glucose/min, and 0.16–0.17 g triglyceride emulsion as 10% Intralipid®-S/min. Observations were made during infusion lasting 4 hr, the second half of which in some subjects was an exercise period.

‡‡Exercise was in the supine position on a cycle ergometer at 50% of the workload, which produced a heart rate of 170/min (W170). It lasted 65–120 min.

subjects exercised and was significant ($p < 0.01$), but in each other experimental category there was a smaller water shift and those shifts were not statistically significant. By contrast, the significant insulin releases were 15–20% of the arterial immunoreactivities. The significant insulin extraction in the fed state exercise group was about 30% of the arterial immunoreactivity.

Relationship Between Ia-cs and Ia

In all experimental categories, significant positive relationships were found between insulin extraction (Ia-cs) and arterial immunoreactivity (Ia) (Table 3). Insulin release was observed below about 20 μU/ml insulin. During exercise in the intravenous fed state, however, extraction was observed at lower Ias.

Relationship Between Ca-cs Substrate and Ia-cs Insulin

Of the carbohydrate and lipid substrates observed, only the extractions of triglyceride and FFA were related to insulin extraction (Table 4). For triglyceride in fasting subjects during exercise, and for FFA in the parenterally fed subjects during exercise, these were negative correlations. The correlations were not dependent on arterial insulin levels. The positive relationships for exogenous triglyceride in the I.V. fed state were no longer evident after partial correlation analysis eliminating the arterial insulin immunoreactivity (rest $r_{1,2,3} = 0.12$; exercise $r_{12.3} = 0.51$).

Table 3. Relationships between arterial-coronary sinus difference in insulin immunoreactivity (Y) and arterial plasma insulin immunoreactivity (X)†

	Fasting		I.V. fed state	
	Rest (21)	Exercise (21)	Rest (36)	Exercise (8)
a	−7.2	−8.9	−2.4	0.3
b	0.33	0.51	0.13	0.29
r§	0.52*	0.54*	0.44**	0.84**

†Regression coefficients for the equation $Y = a + b X$ are shown.

§*r* is the correlation coefficient. Significance is indicated by * ($p < 0.05$), ** ($p < 0.01$.

Table 4. Relationships between extraction of substrates (Ca-cs) and extraction of immunoreactive insulin (Ia-cs) from coronary blood†

	Fasting		I.V. fed state§	
	Rest	Exercise	Rest	Exercise
Glucose	-0.03^{ns} (21)	0.05^{ns} (21)	0.24^{ns} (21)	
Lactate	0.02^{ns} (21)	0.02^{ns} (21)	0.03^{ns} (15)	
Pyruvate	-0.11^{ns} (21)	0.08^{ns} (21)	0.41^{ns} (15)	
FFA	0.05^{ns} (21)	-0.04^{ns} (21)	0.06^{ns} (36)	−0.78* (8)
Triglyceride‡	0.08^{ns} (21)	−0.45* (21)	0.36* (36)	0.82** (8)
Glycerol	0.02^{ns} (21)	0.28^{ns} (21)	0.11^{ns} (21)	

†In each category the correlation coefficient (r) is shown and, beneath it, the number of observations in parentheses. Significance is indicated by ns ($p > 0.05$), * ($p < 0.05$), ** ($p < 0.01$).

§"I.V. Fed State" refers to intravenous infusion of glucose and Intralipid®-S.

‡In the fasting state, triglyceride refers to total plasma triglyceride and in the "I.V. Fed State" to "exogenous triglyceride."

DISCUSSION

These data provide evidence that there are circumstances in which insulin can be taken up and released by the human heart. The observed changes in plasma insulin immunoreactivity across the coronary circulation cannot be accounted for by water shifts. They are unlikely to depend on movement of insulin between formed elements and plasma (Wahlqvist *et al.*, 1972). They could represent redistribution of insulin between vascular tissue and plasma, but even if this is so, presumably, in turn, movement between cardiac muscle cell membranes and extracellular fluid would occur. That coronary sinus immunoreactive insulin is biologically active has not been tested directly, but is suggested by the radioimmunoassay parallelism between standard, arterial, and coronary sinus insulins. Myocardial cells are thought to degrade insulin to a limited extent, however (Forgue and Freychet, 1975).

The arterial plasma insulin appears to be one, but presumably not the only, determinant of insulin uptake and release by the heart. The indices of determination (r^2) (Table 3) were appreciably less than unity. The Ia-cs/Ia relationship was shifted by exercise. Parenteral feeding with glucose and a fat emulsion appears to prevent the extraction of insulin that occurs when insulin levels are elevated by infusion (Tables 1 and 2).

If coronary plasma flow at rest is taken to be 150 ml/min and myocardial mass about 300 g, at an Ia-cs of -2 μU/ml, the heart could release 1 μU/min/g. This could account for about 10% of the total turnover rate of insulin. The reverse situation could apply at elevated plasma insulin levels or during exercise in the parenterally fed state.

The question remains whether or not the uptake and release of insulin observed affect heart metabolism. No relationship to carbohydrate metabolism was recognized. A negative relationship with triglyceride extraction may be consistent with the reciprocity between lipoprotein lipase in adipose tissue, which is activated by insulin, and in the heart (Borensztajn, Samols, and Rubinstein, 1972). The negative relationship between FFA extraction and insulin extraction was unexpected, considering the antilipolytic action of insulin in adipose tissue, but a different situation may prevail in the heart during exercise and where competition between triglyceride and FFA as substrates may occur (Wahlqvist *et al.*, 1974).

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