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Hormone Metabolism in the Pulmonary Circulation

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Summary

We measured hormonal levels in blood samples from pulmonary and radial arteries in 117 patients undergoing aorto-coronary by-pass surgery with the aim of investigating the role of the pulmonary vessel endothelium in hormone metabolism. Insulin and glucagon concentrations were significantly higher in pulmonary artery blood with respect to radial artery blood (73±65 *vs*. 65±47 pmol/l, $p<0.005$, and 80±49 *vs*. 73±51 ng/l, $p<0.01$, respectively), while no difference was found for growth hormone, prolactin, C peptide, insulin-like growth factor I, follicle stimulating hormone, luteinizing hormone, thyroid stimulating hormone, parathyroid hormone, thyroglobulin, triiodothyronine, thyroxine, free triiodothyronine, and free thyroxine. Moreover, prolactin concentrations were more than twice the normal levels, this being an effect of propafol and the opiate fentanyl used for the general anesthesia. Assuming that the arteriovenous differences observed are a marker of peptide hormone degradation, our study has demonstrated that with similar kinetics insulin and glucagon secreted into portal circulation and escaping from hepatic extraction undergo further homeostatic removal of about 9-10 % in the pulmonary circulation before entering the general circulation.

Key words

Peptide hormones • Thyroid hormones • Endothelial cleavage • Lung • Insulin • Glucagon • Peptide degradation

Introduction

In addition to bringing blood into contact with alveolar gas for gas exchange, the lung has been shown to have the capacity for complex metabolic activities. They include the selective removal or inactivation of serotonine, bradykinin, adenine nucleotides and certain prostaglandins, the conversion of angiotensin I to angiotensin II, the synthesis and release of some prostaglandins, thromboxane, prostacyclin, leukotrienes, histamine, peptides and enzymes, such as vasoactive peptides, lymphokines, and plasminogen activator. However, some compounds, including epinephrine, dopamine, thyramine, and peptide hormones, such as substance P, oxytocin, and vasopressin, are not metabolized in the pulmonary circulation (Said 1982).

For many hormones, however, the metabolic role of the lung does not seem to be fully investigated (Rubenstein *et al.* 1968). Since the arteriovenous difference in hormone concentrations may be considered as a marker of its enzymatic degradation (Cuatrecasas *et al.* 1998), we measured the hormonal levels in the serum from pulmonary and radial arterial blood in humans.

Methods

The study was carried out, after informed consent had been obtained, in 117 patients (93 men and 24 women of mean age 59.8±9.5 years), undergoing aorto-coronary by-passes for atherosclerotic coronary disease. Patients with endocrine and metabolic diseases or with renal and liver impairment were excluded. All patients were operated using the same anaesthetic and surgical technique. After sternotomy and before heparin anticoagulation, hypothermia and extracorporeal circulation were introduced, blood specimens were nearly simultaneously withdrawn into sterile syringes from the pulmonary and radial arteries and then collected in red-stoppered vacutainer tubes (Becton-Dickinson Vacutainer Systems Europe, Plymouth, UK), centrifuged and frozen.

By immunoradiometric assay, we measured the human growth hormone (GH), prolactin (PRL), follicle stimulating hormone (FSH), luteinizing hormone (LH) (BioChem. ImmunoSystems Italia, Casalecchio di Reno (Bologna), Italy); thyroid-stimulating hormone (TSH), thyroxine (T_4), triiodothyronine (T_3) and thyroglobulin (TG) (Radim S.p.a., Pomezia (Rome), Italy); parathyroid hormone (PTH; Incstar Corp., Stillwater, Minnesota, USA). We employed radioimmunoassay methods for measuring insulin (IRI, 14 % human proinsulin cross-reaction, 7.17 pmol/l limit of detectability, 5.3 % coefficient of intra-assay variation, 5.5 % coefficient of inter-assay variation), C peptide (C Pept, 0.01 % human insulin cross-reaction), insulin-like growth factor I (IGF I), glucagon (IRG, no cross-reaction, 14.5 ng/l limit of detectability, 8.4 % coefficient of intra-assay variation, 8.6 % coefficient of inter-assay variation) (BioChem ImmunoSystems Italia S.p.a.), free thyroxine (FT_4) and free triiodothyronine (FT_3) (Ortho-Clinical Diagnostics, Amersham, U.K.).

Pulmonary and radial artery blood samples from each patient were measured in the same assay run. The data variability was expressed as standard deviation (SD).

Student' s t-test for dependent samples was used for statistical analysis (Statistica Software, Stat. Soft. Inc. 1993).

Table 1. Hormone levels in serum from human pulmonary and radial arteries

	Normal ranges	**n**	**Pulmonary artery**	**Radial artery**	**P=**
GH	(0.1-5.0 µg/l)	53	1.61±1.94	1.58±1.91	0.41
PRL	(1.0-20 µg/l)	78	57.97±29.25	58.61±28.32	0.52
IGF 1	(144-360 µg/l)	76	138.80±44.31	135.78±45.62	0.34
IRI	(14-179 pmol/l)	107	72.96±64.79	65.29±46.68	0.003*
IRG	(60-200 ng/l)	78	80.15±49.21	72.64±50.81	0.009**
C-Peptide	(1.0-3.0 µg/l)	84	2.69±1.46	2.68±1.49	0.86
FSH°	(1-100 IU/l)	53	7.09±9.65	7.65±9.81	0.34
LH°	(1.5-61 IU/l)	53	3.20±3.10	3.11±2.85	0.23
TSH	(0.25-4.0 mU/l)	65	1.57±1.23	1.58±1.29	0.77
PTH	(10.6-54 ng/l)	45	42.25±23.69	39.39±18.59	0.34
T_3	(0.92-2.91 nmol/l)	41	1.24±0.53	1.27±0.59	0.56
FT_3	(2.76-8.60 pmol/l)	62	5.68±2.87	5.77±3.57	0.51
T_4	(58-142 nmol/l)	41	113.29±33.59	113.68±33.79	0.84
FT_4	(10-24 pmol/l)	62	19.69±14.15	20.72±14.80	0.35
TG	(1-60 µg/l)	40	16.67±29.53	16.67±26.03	0.99

*Data are means ± S.D., ° men and menopausal women; statistical significance : * p<0.005, ** p<0.01.*

Results

The results are summarized in Table 1. Significant differences were observed between the pulmonary and radial artery insulin ($p<0.005$) and glucagon ($p<0.01$) serum levels. The levels of these two hormones from the pulmonary artery were higher compared to those from the radial artery. The 95 % confidence limits of serum insulin values were 60.55-85.45 pmol/l in the pulmonary blood and 56.32-74.26 pmol/l in the radial blood and they were 67.21-93.08 and 59.28-86.00 ng/l for glucagon, respectively. The percentage differences between the means were 10.5 % for insulin and 9.3 % for glucagon.

No significant differences were found for the other hormones investigated. Moreover, PRL levels, both pulmonary and radial, were much higher with respect to the ranges for normal adults.

Discussion

The results of the present study seem to prove that the lungs play a significant role in the metabolism of insulin and glucagon. Their levels in the blood from the radial artery were significantly lower than those found in the pulmonary artery. No differences, however, were found for the other investigated peptide hormones, neither for the thyroid hormones and thyroglobulin.

The enzymatic degradation of insulin and glucagon molecules into fragments not recognized by the antibodies used in the immunoassay seems to be the reason for the differences observed. On the other hand, it is well known that the vascular endothelium cells contain enzymes metabolizing various circulating peptides (Erdos *et al.* 1978), in particular those in the pulmonary circulation. Here, several metabolic activities occur due to the large dimension of the pulmonary vascular bed.

Over 40 % of the insulin secreted from the pancreas is extracted from the portal circulation in a single pass through the liver (Jaspan *et al.* 1981) and insulin-degrading activity has been found in various tissues, the highest activity being present in the kidney and muscles. Nevertheless, it is also present in the pancreas, ovary, brain, testis, spleen, lung, heart and fat homogenates (Kitabchi and Stentz 1972, Authier *et al.* 1996). Moreover, a single report on six patients undergoing cardiac catheterization suggested that insulin may be cleared by the lung (Rubenstein *et al.* 1968). Although numerous enzymes with insulin-degrading activity have been described, thiol-metalloendopeptidases seem to exert the main insulinase activity with a wide tissue distribution and displaying a broad substrate specificity, including glucagon, insulin growth factor II and atrial natriuretic factor (Authier *et al.* 1994, 1996).

Glucagon secreted by the α-cells of the pancreatic islets into the portal circulation is commonly held to act almost exclusively on the liver, undergoing consistent hepatic extraction of about 35 % (Jaspan *et al.* 1981).

Thus, with similar kinetics, insulin and glucagon seem to undergo further homeostatic removal in the pulmonary circulation before they enter the general circulation.

As a matter of fact, endothelial cells are involved in the transport of hormones from the lumen of blood vessels, being the major barrier for the rapid diffusion of hormones to their target cells. Moreover, unlike other cells, endothelial cells of systemic vessels have been demonstrated not to degrade insulin to any extent, transporting the hormone across endothelial cells by a receptor-mediated process (King and Johnson 1985). However, the endothelial cells particularly in the pulmonary circulation should form a barrier to the diffusion of hormones, because there are no target tissues to justify appreciable hormones diffusion, since only the basal membrane and the pulmonary epithelial cell layer are interposed.

For this reason, the observed differences in concentrations of insulin and glucagon between pulmonary and radial arteries seem to be an expression of cleavage, since no such differences were found for the other hormones investigated. On the other hand, mere hormone uptake or diffusion without cleavage would not result in concentration differences in the steady state.

Moreover, the unchanged C peptide concentration after the pulmonary pass agree with its considerably longer half-life, about 30 min, with respect to the equimolarly secreted insulin (4-6 min) or glucagon (4-6 min) and with the almost complete intrapancreatic conversion of proinsulin (Alford *et al.* 1976, Jaspan *et al.* 1981, Robbins *et al.* 1984). In fact, unconverted proinsulin, has very low biological activity and does not seem to be converted to insulin once released into the circulation, being degraded in peripheral tissues with a half-life of about 17 min (Kitabchi and Stentz 1972, Robbins *et al.* 1984). The immediate destruction of peptide C is not as physiologically important as that of insulin and glucagon, probably due to the lack of

biological activity. Furthermore, the proinsulin cross-reaction in the insulin immunoassay does not significantly affect the differences observed.

Despite the structural similarity to insulin, IGF I is very slowly degraded, if at all, by the insulin-degrading enzymes (Roth *et al.* 1984). Furthermore, its levels do not seem to be affected by the pulmonary pass, since it is protected from proteolysis, circulating in the blood as a part of a protein complex. This might impede its transfer across the endothelial wall (Daughaday 1985).

GH, PRL, FSH, LH, thyroid hormones and PTH also do not seem to be metabolized during pulmonary circulation. This might be partly due to a lack of finalism of a pulmonary removal of hormones soon after the homeostatic down-regulation of their circulating levels, before they reaching their target tissues. However, this may not be an explanation for the unchanged TSH and TG levels.

As far as the highly increased PRL levels are concerned, this seems to be secondary to the effect of the opiate fentanyl and of propofol used in the general anesthesia (O' Leary *et al.* 1996, Reber *et al.* 1998).

In conclusion, our study has indicated that insulin and glucagon, secreted by the pancreas into the portal circulation and escaping from hepatic degradation, undergo further homeostatic removal in the pulmonary circulation before entering the general circulation. The mean proteolytic activity of the pulmonary endothelium seems to destroy 10 % of circulating insulin, indicating a pulmonary clearance rate similar to that reported for other peripheral tissues (Samols and Ryder 1961). The mean glucagon extraction rate was almost the same after the pulmonary pass, while no effect was demonstrated for the other hormones investigated.

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