EFFECT OF PHOSPHATE OMISSION ON ARGININE-INDUCED INSULIN AND GLUCAGON RELEASE BY THE ISOLATED PERFUSED RAT PANCREAS

J. E. CAMPILLO+, A. S. LUYCKX, M. D. TORRES and P. J. LEFÈBVRE*
Division of Diabetes, Institute of Medicine, University of Liège, B-4020 Liège, Belgium

Received 28 September 1977

1. Introduction

Over the past few years, the involvement of cations in the secretion of insulin and glucagon from the pancreatic islets has been the subject of growing interest (reviews [1,2]). By contrast, the role of anions in the secretory process of these hormones has received less attention [3–6].

The aim of this work was to investigate the effect of phosphate ($H_2PO_4^-$) on the glucagon and insulin release in response to arginine.

2. Materials and methods

Overnight-fasted male Wistar rats (200–300 g) were utilized in all experiments. Techniques for isolation and perfusion of the rat pancreas have been described [7]. The composition of the perfusate was: NaCl 120 mM; KCl 4 mM; $MgSO_4$ 0.7 mM; $NaHCO_3$ 25 mM; $CaCl_2$ 1 mM, with or without KH_2PO_4 1.2 mM for each experimental condition. In the absence of phosphate, an equivalent amount of KCl was added to avoid variations in the potassium concentration. The perfusate was supplemented with 2% (w/v) bovine albumin (Armour Pharmaceutical) and 2.5% (w/v) of dextran T-70 (Poviet). All experiments were performed in the absence of glucose in the perfusate. The medium was warmed at 38°C and continuously gassed with a mixture of O_2 (95%) and CO_2 (5%). The resulting pH was 7.5. After a 30 min pre-stimulation period, L-arginine–HCl (Fluka AG) was infused through a side-arm perfusion pump (Braun) at a rate calculated to reach a final concentration of 10 mM. The stimulation period lasted 30 min and was followed by a 10 min post-stimulation period in the absence of the amino acid. Samples, 2 ml, were collected into chilled tubes containing 1000 U Trasylol (Bayer) and frozen at −20°C until assayed. Insulin (IRI) and glucagon (IRG) were measured by radioimmunoassay as described [7]. All results are expressed as mean ± SEM and statistical analysis was performed using the Student's *t* test for non-paired data.

3. Results

Both in the presence as in the absence of $H_2PO_4^-$ in the perfusate, arginine 10 mM elicited a biphasic glucagon release and a monophasic insulin secretion (fig.1). In the absence of phosphate, the first phase of glucagon release was unchanged, while the second phase was significantly augmented (fig.1, table 1). Insulin response to arginine was also increased in the absence of phosphate. As illustrated by fig.1, the insulin release was unaffected until 15 min but was significantly augmented from that time onward.

4. Discussion

This study shows that omission of extracellular phosphate ($H_2PO_4^-$) significantly enhances insulin and

+ Permanent address: Departamento de Fisiologia, Faculdad de Medicina, University of Granada, Granada, Spain

* Address correspondence to: Dr P. J. Lefèbvre

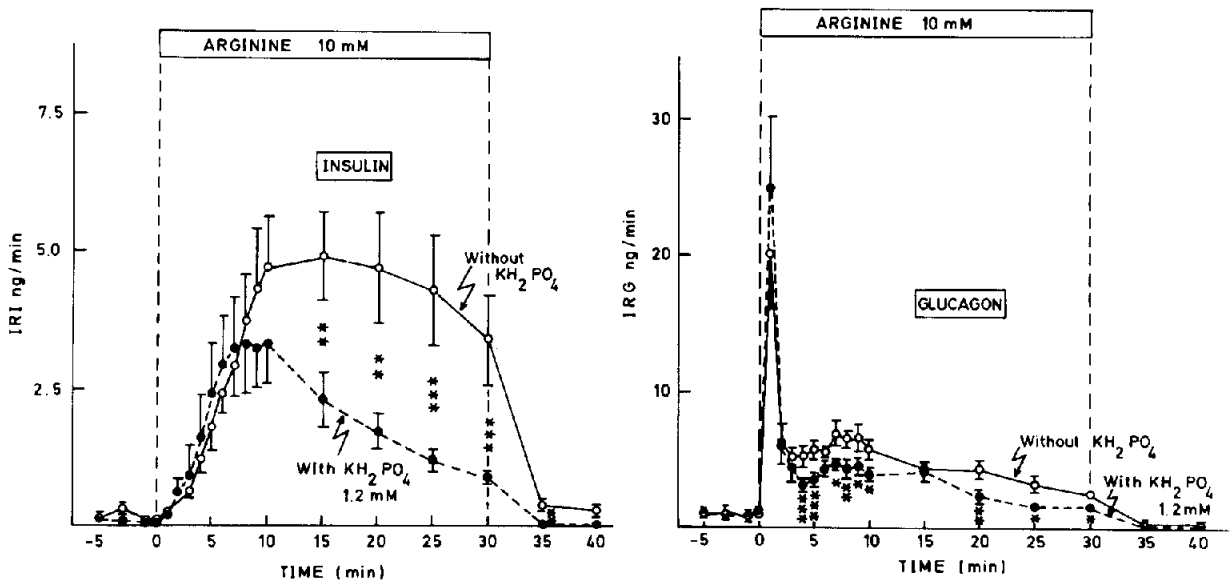

Fig.1. Insulin (IRI) and glucagon (IRG) responses to arginine with complete medium ($n = 8$, •– – – –•) and $H_2PO_4^-$-free medium ($n = 7$, o——o). Results are expressed as mean ± SEM. Statistical comparison corresponds to: *, $p < 0.05$; **, $p < 0.02$; and ***, $p < 0.01$.

glucagon responses to arginine 10 mM in the absence of glucose in the perfusate.

No data are available in the literature concerning the movements and effects of phosphate anions during stimulation of insulin and glucagon release by arginine. It has been reported [3] that a transient ^{32}P efflux from prelabelled pancreatic islets occurs as an early and highly specific response to glucose or other sugars which act as insulin secretagoges, that the glucose-enhanced ^{32}P release shows an anomeric specificity [4], and that it is impaired by starvation [6].

In conclusion, from the results of the present investigation and from the data so far available in the literature, it is suggested that extracellular phosphate anions are involved in the modulation of the insulin and glucagon release in response to a secretagoge. The mechanisms whereby phosphate omission enhances both insulin and glucagon responses to arginine remain to be elucidated.

Table 1
Effect of phosphate ($H_2PO_4^-$) omission on arginine-induced glucagon and insulin release

	Glucagon		Insulin
$KH_2PO_4^-$ (mM)	First phase (ng/3 min)	Second phase (ng/27 min)	Total (ng/30 min)
1.2 ($n = 8$)	35.2 ± 7.0	81.8 ± 8.5	59.2 ± 12.5
0.0 ($n = 7$)	31.2 ± 6.2	122.5 ± 10.5	112.3 ± 21.9
Statistical analysis	NS	$p < 0.01$	$p < 0.05$

n, number of experiments

NS, not statistically significant

Acknowledgements

The authors wish to acknowledge the technical assistance of Mrs M. Marchand and Mrs C. Cartenstadt. This work was supported in part by the Fonds National de la Recherche Scientifique (FNRS) of Belgium. Dr A. S. Luyckx is Maître de Recherches du Fonds National de la Recherche Scientifique (Belgium). Dr J. E. Campillo is a Visiting Scientist from the University of Granada.

References

[1] Randle, P. J. and Hales, C. N. (1972) in: Handbook of Physiology, Section 7: Endocrinology: Endocrine Pancreas (Steiner, D. F. and Freinkel, N. eds) pp. 219–235, American Physiological Society, Washington D.C.
[2] Leclerq-Meyer, V., Marchand, J. and Malaisse, W. J. (1976) Horm. Res. 7, 348–362.
[3] Freinkel, N., El Younsi, C., Bonnar, J. and Dawson, R. M. C. (1974) J. Clin. Invest. 54, 1179–1189.
[4] Pierce, M. and Freinkel, N. (1975) Biochem. Biophys. Res. Commun. 63, 870–874.
[5] Henquin, J. C. and Lambert, A. E. (1976) Am. J. Physiol. 231, 713–721.
[6] Asplund, K. (1976) in: Current Topics in Diabetes Research (Bajaj, J. S. ed) p. 2, Excerpta Medica Int. Congr. Ser. No. 400, Amsterdam.
[7] Campillo, J. E., Luyckx, A. S., Torres, M. D. and Lefèbvre, P. J. (1977) submitted.