2 ng/g wet tissue. The plasma level of SP was 180 pg/ml. The normal level of SP is 41±7 (SEM) pg/ml[4].

The pathophysiology of the carcinoid syndrome in MCT is unknown and the elevation of 5-hydroxyindole-acetic acid in urine, if present, is usually slight[11]. Prostaglandins and kallikrein have also been implicated in the genesis of the carcinoid syndrome but the evidence is inconsistent[11]. SP accompanies 5-HT in enterochromaffin cells[12]. In human volunteers, ng-quantities of SP infused parenterally induced carcinoid-like flush, hypotension, and increased intestinal motility[13]. The biological effects of SP suggest the possibility that SP may contribute to the pathophysiology of the carcinoid syndrome in MCT or carcinoid tumours. The finding of SP in MCT and in carcinoid tumours support the concept that MCT and carcinoid tumours are related[14].

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Effect of phosphate omission on glucose-induced insulin release in vitro

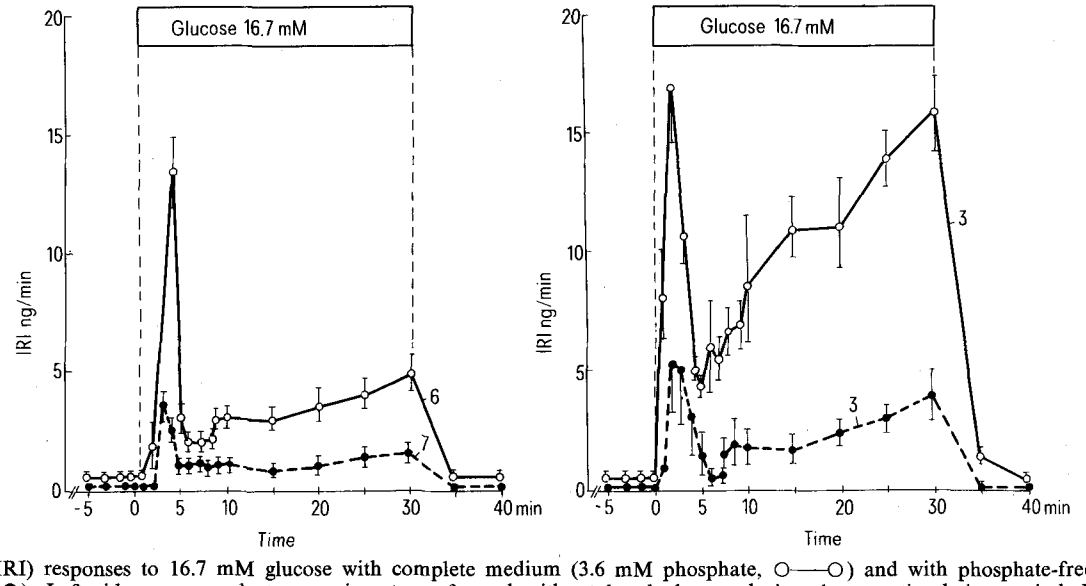
J.E. Campillo, M. Castillo, M.M. Valdivia and E. Rodriguez

Departamento de Fisiologia y Bioquimica, Facultad de Medicina, Universidad de Granada, Granada (Spain), 14 December 1978

Summary. In the isolated perfused rat pancreas, omission of extracellular phosphate ($H_2PO_4^-$) significantly reduces the insulin secretion in response to 16.7 mM glucose.

Increasing evidence suggests that phosphate anions could play an important role in the mechanism of insulin release from the stimulated beta cell[1-9]. This study aimed at investigating the effect of extracellular phosphate ($H_2PO_4^-$) on the insulin response to 16.7 mM glucose.

Materials and methods. Overnight fasted male wistar rats (200-250 g) were utilized. Techniques for isolation and perfusion of the rat pancreas have been described[10]. The perfusate contained: NaCl 120 mM; KCl 1.4 mM; $MgSO_4$ 0.7 mM; $NaHCO_3$ 25 mM; $CaCl_2$ 1.0 mM; with or without 3.6 mM KH_2PO_4 for each experimental condition. In the absence of KH_2PO_4, an equivalent amount of KCl was added to avoid variations in the potassium concentration. The perfusate was supplemented with 0.5% (w/v) bovine



Insulin (IRI) responses to 16.7 mM glucose with complete medium (3.6 mM phosphate, O——O) and with phosphate-free medium (●– – –●). Left side corresponds to experiments performed without basal glucose during the pre-stimulation period. Right side corresponds to experiments performed in the presence of 2.7 mM basal glucose. Number of experiments are indicated with arrows. Results are expressed as mean ± SEM.

Effect of phosphate omission on the glucose-induced insulin (IRI) release

$H_2PO_4^-$ (mM)	Without basal glucose		With 2.7 mM basal glucose	
	1st phase (ng/5 min)	2nd phase (ng/25 min)	1st phase (ng/5 min)	2nd phase (ng/25 min)
3.6	27.2±4.7	84.3±8.9	45.5±5.3	277.3±49.2
0.0	7.6±1.4	27.4±6.8	18.1±9.2	56.2±21.3
Statistical analysis	$p<0.01$	$p<0.01$	NS	$p<0.01$

Number of experiments as indicated in the figure. NS, not statistically significant.

albumin (Armour), and 2% (w/v) dextran T-70 (Ibys). The experiments were performed both in the absence and in the presence of 2.7 mM glucose in the perfusate during the pre-stimulation period. The medium at 37 °C was continuously gassed with O_2 and CO_2 (95:5). After a 30-min pre-stimulation period, glucose (Merck) was infused through the pancreas using a side-arm perfusion pump at a rate calculated to reach a final concentration of 16.7 mM in the perfusate. The stimulation period lasted 30 min. Samples (2 ml) were collected into chilled tubes and frozen at −20 °C until assayed. Insulin (immunoreactive insulin, IRI) was measured as described[10]. All results are expressed as mean ± SEM and statistical analysis was performed using Student's t-test.

Results and discussion. Both in the presence as in the absence of phosphate in the medium, 16,7 mM glucose elicited a biphasic insulin secretion (figure). At 3.6 mM phosphate, the presence of 2.7 mM glucose in the perfusate during the pre-stimulation period, clearly potentiated the insulin secretion in response to 16.7 mM glucose. In the absence of phosphate, both 1st and 2nd phases of insulin release were significantly reduced (table).

Furthermore, in the absence of phosphate, the potentiation of the 16.7 mM glucose-induced insulin release by 2.7 mM basal glucose was severely impaired. These results are in contrast to previous reports which show that the absence of phosphate increased the arginine-induced insulin release when compared to controls with 1.2 mM phosphate[6]. Using the isolated islets from ob/ob mice, it has been reported that phosphate acted as an inhibitor of the glucose-induced insulin release[8]. The mechanism whereby phosphate influences insulin secretion remains to be elucidated. Several reports show that stimulation with insulin secretagogues may trigger a rapid, transient efflux of 32-P from pre-labelled pancreatic islets[1-5]. In conclusion, from the present investigation and from the data so far available in the literature, it is established that extracellular phosphate anions could be involved in the modulation of insulin release from the stimulated beta-cell.

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Measurement of urinary steroid production rates using stable-isotopes and GC-MS

D.W. Johnson, G. Phillipou[1], I.A. Blair and R.F. Seamark

Endocrine Laboratory, Department of Obstetrics and Gynaecology, The Queen Elizabeth Hospital, Woodville 5011 (South Australia), 15 January 1979

Summary. The urinary production rate of pregnenolone has been determined for a male subject using 7,7-d_2-pregnenolone as an isotopic tracer.

The recent general availability of deuterated steroids[2] in high isotopic purity offers the possibility of developing practical clinical procedures for the determination of urinary steroid production rates. These procedures should overcome most of the methodological and hazard drawbacks and also provide more precise estimates than those previously reported using radioactive tracers. The contention is supported by the following study in which the measurement of the urinary pregnenolone production rate is made using a stable-isotope steroid analogue.

A sterile solution of 7,7-d_2-pregnenolone[2] (0.8 mg ml^{-1}; propylene glycol: water, 3:1) was injected into the antecubital vein of a male subject (age 41 years) and urine collection instituted 2 days prior and 3 days post administration of the dose. Aliquots (10 ml) of the total urine collections were specifically hydrolysed (solvolysis or β-glucuronidase, ex coli) to release free steroids from the sulphate and glucuronide fractions respectively. Previous studies have shown that steroid recoveries by these procedures are >80%[3,4]. The required steroids 5-pregnene-3β,20α-diol (5PD) and 5β-pregnane-3α,20α-diol (PD) were further purified by TLC and derivativatized to form the corresponding pentafluoroacyl derivatives[5,6]. GC-MS employing selected ion monitoring[7] was then used to measure the enrichment factors ($ef = d_2(d_0+d_2)^{-1} \cdot 100$) for both 5-PD (M/z 446, 448) and PD (M/z 448, 450). The results are contained in the table.

No measurable quantity of urinary pregnenolone could be found in the samples and accordingly the enrichment factor was measured for 5-PD as this steroid has been indicated in previous studies[8,9] to satisfy the criterion as a 'unique' metabolite of pregnenolone.