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The Stimulus-Secretion Coupling of Glucose-induced Insulin Release

XLIV. A Possible Link between Glucose Metabolism and Phosphate Flush

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Summary. Above a threshold of 3.0–4.2 mmol/l, D-glucose provoked a transient increase in ^{32}P fractional outflow rate from rat pancreatic islets prelabelled with ^{32}P-orthophosphate. Nutrients which stimulate insulin release in the absence of glucose, α-ketoisocaproate and L-leucine, also provoked a phosphate flush. No flush occurred in islets exposed to non-insulinotropic nutrients (L-glutamine and L-lactate) or non-nutrient secretagogues (arginine, tolbutamide, theophylline). A late increase in ^{32}P fractional outflow rate was observed in Ca^{2+} deprived islets stimulated with $BaCl_2$ and theophylline. The occurrence of a phosphate flush did not appear to be attributable to changes in insulin release, cyclic AMP content, membrane polarisation, K^+ conductance, or reduced pyridine nucleotide content. The ^{32}P response to glucose was slightly decreased in the absence of extracellular Ca^{2+} or HCO_3^-, markedly impaired in the absence of K^+, and virtually abolished in the presence of menadione (10 μmol/l). It is proposed that the occurrence of a phosphate flush is linked to the metabolism of nutrient secretagogues, possibly via an increase in O_2 uptake and the production rate of NAD(P)H and ATP.

Key words: Isolated rat islets, phosphate flush, glucose, α-ketoisocaproate, leucine, glutamine, lactate, quinine, menadione, antimycin A, barium, potassium, bicarbonate, calcium, theophylline, tolbutamide, arginine, anoxia, cyclic AMP, insulin release.

Glucose is known to affect the fluxes and content of several ions (Cl^-, H^+, Na^+, K^+, Ca^{2+}) in pancreatic islets cells [1–3].

Freinkel and coworkers reported that glucose also causes a transient increase in inorganic phosphate release from the islets [4]. Such a phosphate "flush" is triggered by D-glucose but not L-glucose [4] and by the α rather than β anomer of D-glucose [5]. It can also be elicited by D-mannose [4] and D-glyceraldehyde [6] but not by D-galactose or D-fructose. Mannoheptulose [7] and 2-deoxyglucose [8] block the glucose-mediated phosphate flush. Leucine and one of its non-metabolisable analogues (BCH) both elicit a phosphate flush [7]. The dose-response relationship of the glucose-induced phosphate flush is somewhat shifted to the left in comparison with data for glucose-induced insulin release [9]. Activation of the phosphate flush by glucose is significantly altered by fasting [10]. The phosphate flush can be dissociated (for example by Ni^{2+}) from the augmented release of ^{45}Ca and insulin evoked by glucose [11]. Virtually all the released radioactivity consists of ^{32}P-orthophosphate [12]. Histochemical and microprobe examination of B-cells show accumulation of inorganic phosphate adjacent to the plasmalemma and nucleolus, which is lost during stimulation by glucose [13]. Freinkel and coworkers consider that the phosphate flush reflects an early event in the sequence of stimulus-secretion coupling, such as the recognition of nutrient stimuli at the cell surface [6]. They acknowledge, however, that the mechanism by which this effect occurs has not yet been fully elucidated. In the present study, we have investigated the possible causal link between biochemical and biophysical events evoked by glucose in the islet cells and the occurrence of the phosphate "flush".

Material and Methods

^{32}P-orthophosphate was purchased from the Radiochemical Centre (Amersham, U.K.); menadione, antimycin A, α-ketoisocaproate, L-glutamine, L-lactic acid, quinine hemisulphate and bovine albumin from Sigma Chemical (St. Louis, Missouri,

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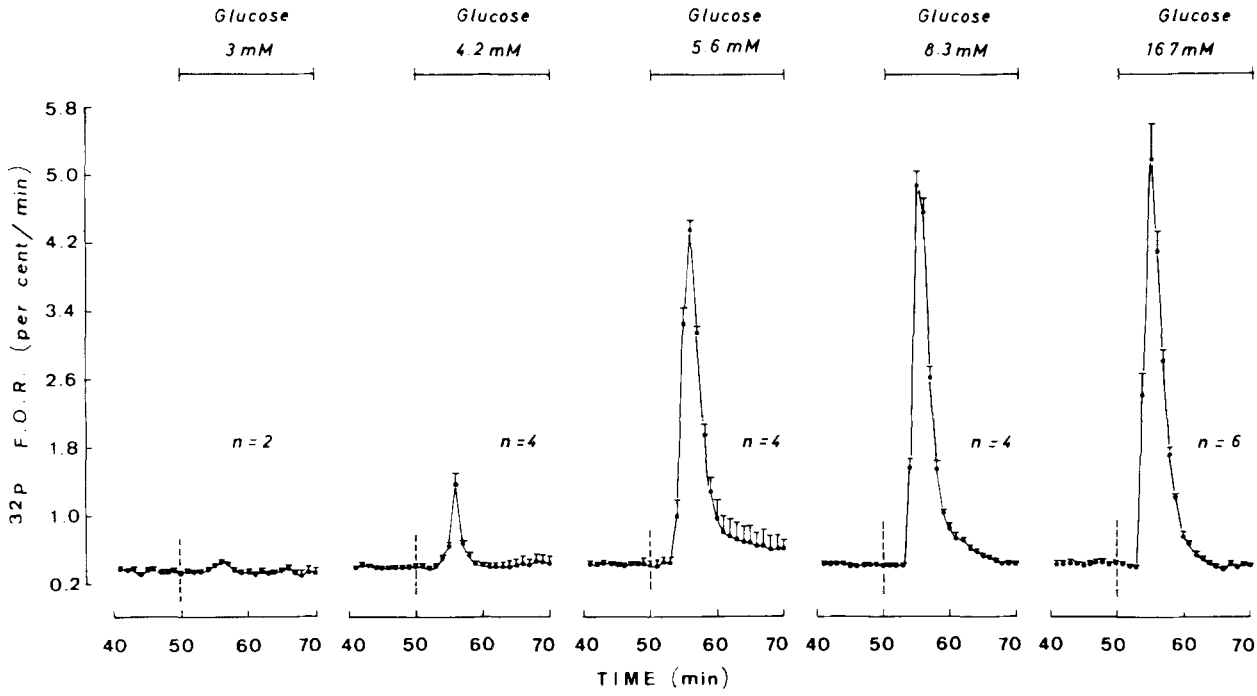

Fig. 1. Effect of an increase in glucose concentration (from zero to the stated value) upon ^{32}P FOR (^{32}P fractional outflow rate) from perifused islets (n = number of individual experiments)

USA); dimethylsulphoxide and dinitrophenol from BDH Chemicals (Poole, U. K.); theophylline from Schwartz/Mann (Orangeburg, New York, USA); and tolbutamide from Hoechst (Frankfurt, Germany)

In each experiment, a group of 100 islets obtained from fed female albino rats [14] was preincubated for 90 min in a bicarbonate-buffered [15] medium (0.2–0.5 ml; pH 7.4) containing bovine albumin (0.5 mg/ml), glucose (16.7 mmol/l) and ^{32}P-orthophosphate (0.5 mmol/l; 7.4 MBq/ml). The islets were washed 6 times with a nonradioactive medium and placed in a perifusion chamber. The perifusate consisted of a buffered solution (pH 7.4) containing albumin (0.5 mg/ml), Na^+ 141, K^+ 5, Ca^{2+} 1, Mg^{2+} 1, HCO_3^- 24, HPO_4^{2-} 1 and Cl^- 124 mmol/l, and equilibrated against a mixture of CO_2 (5%) and O_2 (95%). The perifusate was delivered at a rate of 1 ml/min, the effluent being collected over successive periods of 1 min each and examined for its radioactive content by the Cerenkov counting technique.

Alterations in the composition of the perifusate were achieved as follows. To induce anoxia, the gas phase was replaced by a mixture of CO_2 (5%) and N_2 (95%). For perifusion in the absence of HCO_3^-, the perifusate consisted of a Hepes buffer (25 mmol/l; pH 7.4) containing Na^+ 135 and Cl^- 134 mmol/l (all other ionic concentrations being unchanged) and equilibrated against ambient air. To cause intracellular acidosis, the medium was equilibrated against a mixture of CO_2 (30%) and O_2 (70%) and its concentration of Cl^- (46 mmol/l) and HCO_3^- (102 mmol/l) changed to maintain both the usual pH (7.4) and isoosmolarity (16). When the concentration of K^+ was increased from 5 to 20 mmol/l, the Na^+ concentration was decreased accordingly to maintain isoosmolarity. Menadione and antimycin A were added from stock solutions prepared in dimethylsulphoxide, the latter solvent being used at the same final concentration (0.3–1.7 µl/ml) both during the control and experimental period of perifusion. The perifusate also contained as required glucose, α-ketoisocaproic acid (α-KIC), L-leucine, L-glutamine, L-lactic acid, arginine, tolbutamide, theophylline, dinitrophenol, $BaCl_2$, and quinine hemisulphate. In one series of experiments it was deprived of $CaCl_2$ and contained ethyleneglycol (amino-ethyl ether) tetra-acetic acid (EGTA) (0.5 mmol/l).

The efflux of ^{32}P was expressed as an instantaneous fractional outflow rate (FOR), taking into account the cumulated values of effluent radioactivity and the final radioactive content of the islets. Thus the ^{32}P FOR represents the outflow of ^{32}P (cpm released per min) expressed in percent of the radioactive content of the islets at the same time (min n). The latter content was taken as the sum of the final radioactive content of the islets and the cumulated values of effluent radioactivity (from min n up to the end of the experiment). The final radioactive content of the islets, expressed in terms of inorganic phosphate with the same specific activity as that of the preincubation medium, averaged 13.2 ± 0.7 pmol/islet (n = 57). All data are expressed as the mean ± SEM. In all figures, the vertical dotted line corresponds to the time at which the perifusate was derived from a different reservoir (at the 51st min). The data are not corrected for the dead space which, in our system, imposes a 1–2 min delay between the moment at which the perifusate is derived from a different reservoir and the moment at which the new medium reaches the collecting vial.

Results

1. Effect of Nutrients

The ^{32}P FOR averaged 0.40 ± 0.01% (n = 46) after 50 min perifusion in a glucose-free perifusate. The ^{32}P FOR was little affected when the perifusate administered up to 50 min contained glucose at low

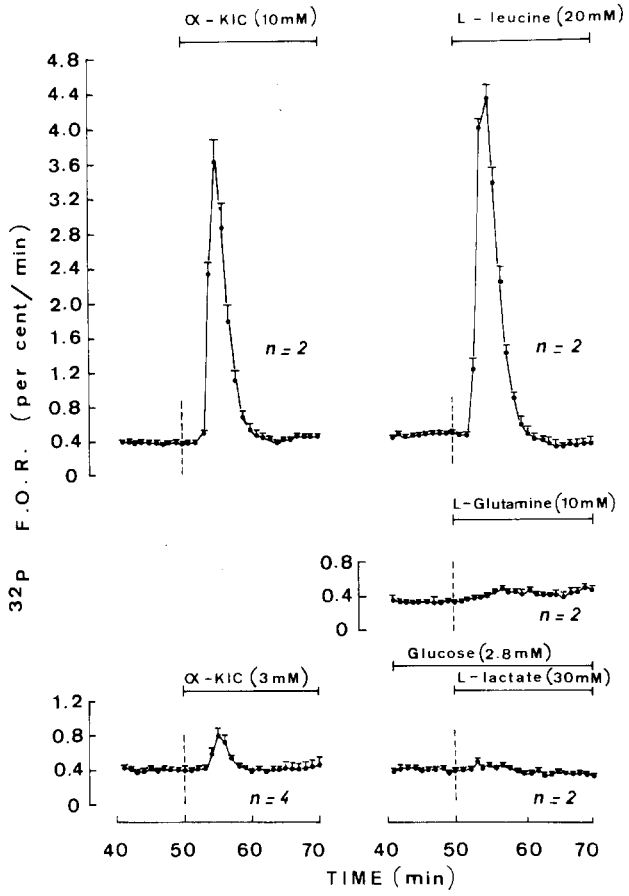

Fig. 2. Effect of α-ketoisocaproate (α-KIC), L-leucine, L-glutamine and L-lactate upon ^{32}P FOR from perifused islets. Whenever the perifusate contained glucose, the same concentration was used throughout the perifusion period

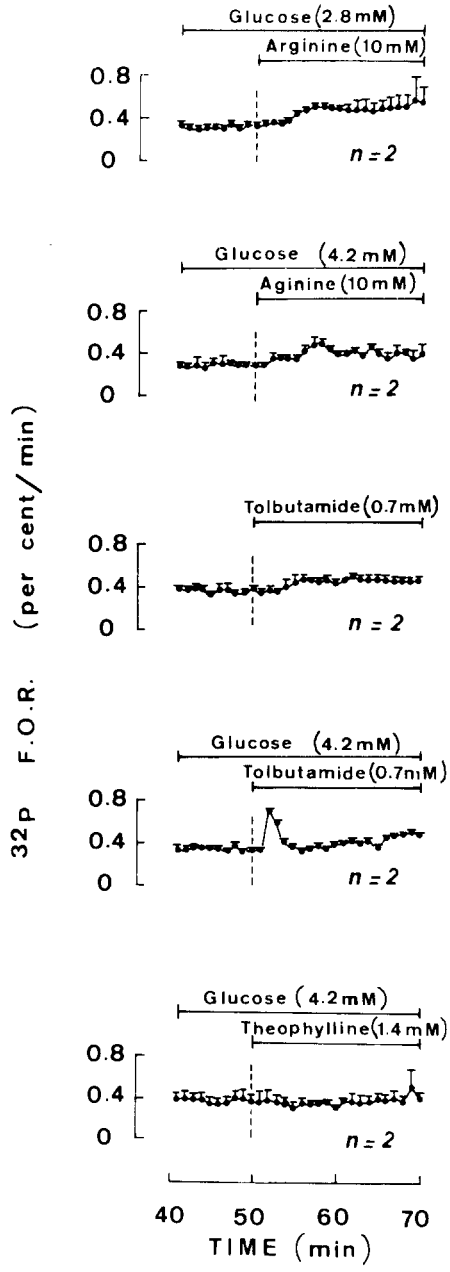

Fig. 3. Effect of arginine, tolbutamide and theophylline upon ^{32}P FOR from perifused islets. Whenever the perifusate contained glucose, the same concentration was used throughout the perifusion period

concentrations, averaging 0.35 ± 0.02 (n = 8) and 0.34 ± 0.02 (n = 12) % after 50 min perifusion in the presence of glucose 2.8 and 4.2 mmol/l, respectively. When the glucose concentration of the perifusate was raised from zero to 4.2 mmol/l or more, a dramatic but short-lived increase in ^{32}P FOR was noted (Fig. 1). The threshold concentration of glucose required to provoke such a phosphate flush was between 3.0 and 4.2 mmol/l. As judged from the peak value in ^{32}P FOR, which was always reached at the 55th or 56th min of perifusion, the dose-action relationship for the response to glucose was sigmoidal with a half-maximal response reached at a glucose concentration close to 4.9 mmol/l. At the highest glucose concentration (16.7 mmol/l), the phosphate flush corresponded to an integrated FOR of 20.1 ± 1.0% (n = 6) over a period of 10 min (54th to 63rd min).

The keto acid α-ketoisocaproate (10 mmol/l) and the amino acid L-leucine (20 mmol/l) were selected as nutrients which are able to stimulate insulin release in the absence of glucose [17, 18]. They both provoked an obvious phosphate flush (Fig. 2, upper panel), which differed from that evoked by glucose by a more rapid onset. This keto acid also affects other variables of islet function, for example O_2 uptake and insulin release, more rapidly than glucose [19, 20]. When α-ketoisocaproate was tested at a 3.0 mmol/l concentration, a modest and transient increase in ^{32}P FOR was observed.

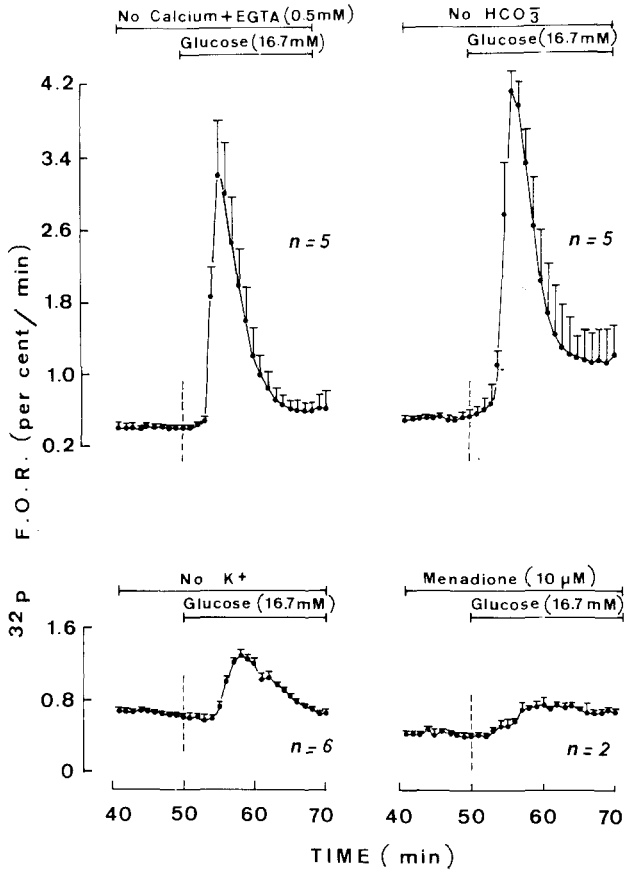

Fig. 4. Effect of an increase in glucose concentration from zero to 16.7 mmol/l upon ^{32}P FOR from islets exposed throughout the 70 min period of perifusion to media either deprived of Ca^{2+} (and containing ethyleneglycol-bis-(β-amino-ethyl ether)NN,N′-tetra-acetic acid; EGTA), HCO_3^- or K^+, or containing menadione (10 μmol/l)

L-glutamine (10 mmol/l) and L-lactate (30 mmol/l) were selected as nutrients which, although well metabolised in the islets, fail to stimulate insulin release in the absence of glucose or at low glucose concentrations [21, 22]. Neither of the two nutrients provoked any obvious change in ^{32}P FOR (Fig. 2).

2. *Effect of Non-nutrient Secretagogues*

Arginine (10 mmol/l), tolbutamide (0.7 mmol/l) and theophylline (1.4 mmol/l) are well known insulinotropic agents. Tolbutamide is able to stimulate insulin release in the absence of glucose [23]. It is debatable whether such is also the case for arginine [24] and theophylline [25] which, however, markedly enhance insulin release in the presence of a low concentration of glucose (4.2 mmol/l). Both in the absence and presence of glucose 4.2 mmol/l, none of these agents caused any marked increase in ^{32}P FOR (Fig. 3). At the most, there might have been a minor response to tolbutamide when the hypoglycaemic sulphonylurea was administered to islets already exposed to glucose 4.2 mmol/l.

3. *Alteration of the Response to Glucose*

The phosphate flush evoked by glucose (16.7 mmol/l) was slightly but significantly reduced ($P < 0.05$) when the islets were perifused in a media deprived of either Ca^{2+} or bicarbonate (Fig. 4, upper panels). The response to glucose was dramatically reduced ($P < 0.001$) in K^+-deprived islets and virtually abolished when the perifusate administered throughout the experiment contained menadione 10 μmol/l (Fig. 4, lower panels).

4. *Effect of Changes in Intracellular pH, Cell Polarization, K^+ Conductance and Redox State*

An increase from 5 to 30% (v/v) in the CO_2 content of the gas phase against which the perifusate is equilibrated causes intracellular acidification in the islet cells [16]. This procedure failed to cause any rapid change in ^{32}P FOR (Fig. 5, upper left panel). A rise in extracellular K^+ concentration from 5 to 20 mmol/l, which causes B cell depolarisation [26] and considerably facilitates Ca^{2+} influx into the islet cells [27], also failed to reproduce the effect of glucose in provoking a phosphate flush (Fig. 5, middle left panel). Quinine (0.1 mmol/l), which reduces K^+ conductance to the same extent as glucose [28], failed to affect ^{32}P FOR (Fig. 5, lower left panels). Exposure of the islets to a perifusate equilibrated with a CO_2–N_2 mixutre (5–95, v-v), a procedure which increases the NAD(P)H content of the islets [29] failed to affect ^{32}P FOR, whether in the absence or presence of glucose (Fig. 5, upper right panels). Likewise, antimycin A which also increases the NAD(P)H content and concomitantly lowers ATP content of the islets [29], failed to provoke any rapid changes in ^{32}P FOR (Fig. 5, lower right panels).

5. *Effect of Barium and Dinitrophenol*

The administration of $BaCl_2$ and theophylline to Ca^{2+}-deprived islets caused a delayed increase in ^{32}P FOR, which occurred after 20–25 min exposure to the secretagogues (Fig. 6). Control experiments indicated that such a late increase was not observed either when theophylline alone was administered to the Ca^{2+}-deprived islets, or when dinitrophenol was administered in order to reproduce the fall in ATP content occurring in Ca^{2+}-deprived islets exposed to $BaCl_2$ and theophylline [30].

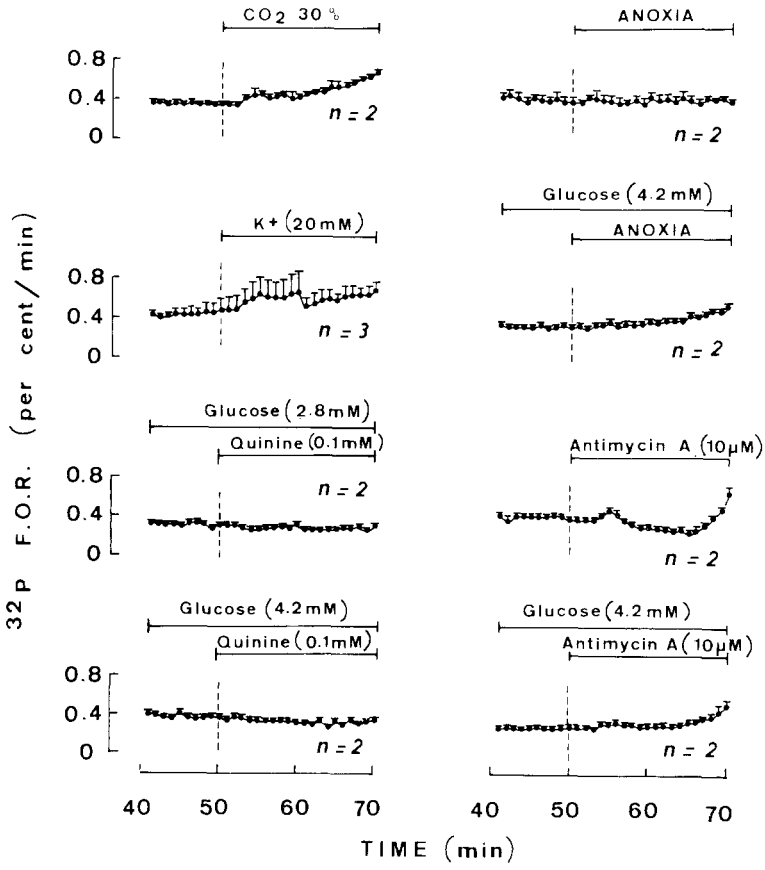

Fig. 5. Effect of a change in CO_2 content of the gas phase (from 5 to 30%; v/v), of an increase in K^+ concentration (from 5 to 20 mmol/l) and of quinine (0.1 mmol/l), anoxia or antimycin A (10 μmol/l) upon ^{32}P FOR from perifused islets. Whenever the perifusate contained glucose, the same concentration was used throughout the perifusion period

Discussion

The present data suggest that a phosphate flush occurs whenever the B-cell is stimulated by nutrient secretagogues but does not occur when the B-cell is exposed to either non-insulinotropic nutrients of non-metabolisable secretagogues.

Obviously, the phosphate flush is not secondary to insulin release. It occurred when the secretory response was abolished by using a perifusate deprived of extracellular Ca^{2+}. The fact that the concentrations of glucose required to provoke a phosphate flush (threshold value between 3.0 and 4.2 mmol/l) and to evoke a half-maximal response in ^{32}P FOR (glucose 4.9 mmol/l) were much lower than the corresponding values for insulin release [31] also indicates that the phosphate flush is not merely the consequence of insulin release. The dose-action relationship here observed is in good agreement with the data reported by Pierce et al. [9]. In the case of α-ketoisocaproate also, the ^{32}P response occurred at a concentration lower than that required to stimulate insulin release [17].

The sequence of events leading to stimulation of insulin release by glucose is tentatively viewed as follows. The metabolism of glucose generates coupling factors (for example, reduced pyridine nucleotides, high-energy phosphate intermediates, protons) which may affect the movements of ions across membrane systems in the islet cells. The change in K^+ [3] and possibly Cl^- [32] conductance leads to a slow depolarisation and, once the membrane potential reaches a threshold value, this causes the gating of voltage-dependent Ca^{2+} channels [33]. Glucose may also affect the outflow rate [34] and intracellular distribution [35] of Ca^{2+}. The resulting accumulation of Ca^{2+}, presumably in the cytosol or its ectoplasmic compartment, activates both adenylate cyclase via calmodulin [36] and the microbutular-microfilamentous effector system controlling the translocation and exocytosis of secretory granules [37]. In the present work, we have attempted to identify the factor(s) in this sequence responsible for initiation of the phosphate flush.

Our data suggest that none of the following factors is sufficient to evoke a phosphate flush: intracellular acidification (as caused by an increase in pCO_2), decrease in K^+ conductance (as caused by quinine), membrane depolarisation (as caused by high K^+), accumulation of cyclic AMP (as caused by theophyl-

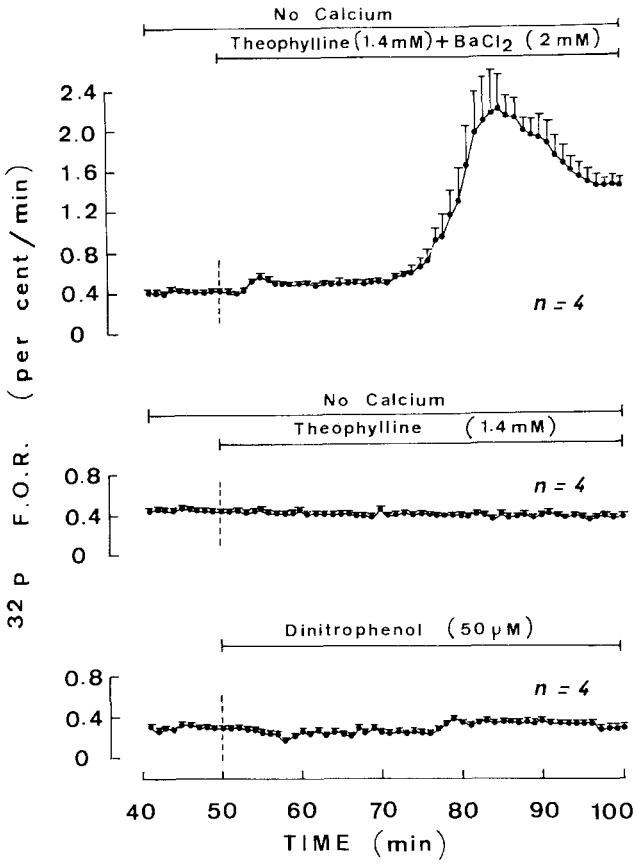

Fig. 6. Effect of theophylline (1.4 mmol/l) with or without $BaCl_2$ (2 mmol/l) upon ^{32}P FOR from islets exposed throughout the 100 min period of perifusion to a medium deprived of calcium and glucose (upper panels). Effect of dinitrophenol (50 μmol/l) upon ^{32}P FOR from islets exposed throughout the 100 min period of perifusion to a medium containing glucose (3.5 mmol/l; lower panel)

line) and induction of a more reduced redox potential (as caused by either anoxia or antimycin A). The phosphate flush seems dependent, however, on the integrity of glucose metabolism. It was markedly reduced in K^+-deprived islets, in which glycolysis is severely impaired [38]. It was virtually abolished by menadione, in the presence of which glucose fails to increase the islet content of NADH and NADPH [39]. It was slightly dimisnished in Ca^{2+}-deprived islets, in which the metabolism of glucose is also slightly decreased [40, 41], and in the absence of bicarbonate, which decreases both basal and glucose-stimulated O_2 uptake by the islets [20].

It would thus appear that the phosphate flush is directly related to some metabolic event(s) evoked by glucose or other insulinotropic nutrients in the islet cells. For instance, it is conceivable that the phosphate flush occurs as a consequence of an increase in O_2 uptake coupled with an increased generation rate of both NAD(P)H and ATP. Such a hypothesis is compatible with the failure of glutamine and lactate to provoke a phosphate flush since these nutrients, in contrast to glucose [29], α-ketoisocaproate [17], and leucine [18], have little or no effect upon the NAD(P)H content of islets deprived of glucose [21, 22]. The hypothesis is also compatible with the threshold concentrations here found for the glucose-stimulated (3.0–4.2 mmol/l) and α-ketoisocaproate-induced (< 3.0 mmol/l) phosphate flush, these concentrations being close to those required to augment O_2 uptake by the islets [20]. Last, the hypothesis could account for the late increase in ^{32}P FOR found in Ca^{2+}-deprived islets stimulated with $BaCl_2$ and theophylline. Indeed in such islets, the utilisation of eendogenous nutrients is significantly enhanced [30]; the rate of insulin release is also higher than that evoked by the other non-nutrient secretagogues tested in the present study (that is arginine, tolbutamide, theophylline, high K^+ and quinine).

In conclusion, the present data, taken together with previous reports on the regulation of phosphate outflow from the islet cells [4–13], suggest that the occurrence of a phosphate flush, like other ionic events, may be closely linked to and depend on the metabolic response of the islets to insulinotropic nutrients.

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