# Lifting the Veil on the "Phosphate Flush," a Cryptic Phenomenon of Experimental Pancreatic Islet Physiology

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The original encounter of the "phosphate flush" in isolated pancreatic islets by Norbert Freinkel (1,2) is steeped in the history of research into the complexities of "stimulus secretion coupling" in cells and organs of the body (3), in particular of the discovery of the "phosphoinositide effect" in studies by Mabel and Lowell Hokin (4). The publication by Berggren and colleagues discussed here (5) can be traced to and must be understood on this pioneering background. The discovery of the phosphate efflux channel XPR1 in pancreatic β-cells and first success of describing its regulation provide new fundamental insights into the role of cellular phosphate and inositol-phosphate metabolism in pancreatic islet physiology.

The "phosphate flush" is a large efflux of inorganic phosphate (i.e., ∼50% of the cellular content) within a 10-min period induced by high glucose or other suitable fuel stimuli (e.g., leucine) under highly specific experimental conditions. The phenomenon can be dissociated from stimulation of insulin release (1). It is demonstrable either by measuring release of radioactive phosphorous from prelabeled cells or by the net loss of inorganic phosphate ($P_i$) from cells using biochemical analytical methods (6,7). To observe it, isolated islets or suitable β-cell lines are preconditioned for extended periods at low glucose levels, most often 0.5 mmol/L but sometimes 2.8 or 3 mmol/L. These conditions are known to result in a low-energy state (a much decreased ratio of ATP/ADP × $P_i$) and, through equilibrium of adenylate kinase ($K = ATP \times AMP/ADP^2$), greatly increased levels of AMP in β-cells (6,7). The intracellular phosphate concentration readily doubles due to hydrolysis of high-energy phosphate bonds, including breakdown of phosphocreatine (P-Cr), conversion of ATP to ADP and AMP and further degradation of AMP by AMP deaminase. There are other potential endogenous sources of releasable phosphate (8) keeping in mind that phosphate is the major intracellular anion. This striking elevation of intracellular $P_i$ is confirmed in the current study. Importantly, a $P_i$ flush—although reduced (1)—can be observed in the absence of extracellular $P_i$ (6,7) and therefore does not require concurrent uptake from the extracellular medium by sodium-dependent transport (9). Once the islet cells have attained this energy-depleted state, acute exposure to a high concentration of glucose or another fuel stimulus results in rapid recovery of their energy state clearly manifest by a rapid drop of AMP and restitution of ATP and P-Cr levels. Regulatory mechanisms are concurrently activated, and any excess intracellular $P_i$ is exported from the cells. If, however, the islet cells are provided with sufficient oxidizable metabolites, such as a combination of 4 mmol/L glucose and a physiological mixture of amino acids, the energy state is not severely depleted. Under these conditions, acute exposure to a higher fuel stimulus of release does not result in efflux of $P_i$ (10). When the energy state was so maintained, there was only a decrease in free MgADP observed from 45 to 30 μmol/L, apparently sufficient to trigger secretion. The above considerations and data illustrate that a "phosphate flush" is only observable under unphysiological experimental conditions. Still, this experimental phenomenon reveals that regulation of phosphate efflux must be considered as a significant factor in maintaining intracellular phosphate homeostasis.

Several recent studies (11,12) have demonstrated that phosphate efflux from mammalian cell lines occurs predominantly through the unique inositol pyrophosphate–controlled anion channel XPR1 (for xenotropic and polytropic retrovirus receptor 1). In the current study, Berggren and colleagues tested the attractive hypothesis that this channel might also mediate the fuel-induced "Pi flush" from islet β-cells and that phosphoinositide-derived, stimulatory second messenger molecules IP7 and/or IP8 might be the responsible coupling factors. Through testing this hypothesis, the Stockholm group closes the long historical loop of investigations into the interrelated "PI effect" and "phosphate flush" (1,2,4,13). Their experiments convincingly demonstrate that this channel is present in a β-cell line and an islet preparation and is essential for the fuel-elicited

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"$P_i$ flush." They also show that suppression of enzymes crucial for generating IP7 and IP8 (11,12) increases intracellular phosphate consistent with an XPR1 activator role of these second messengers. But although inositol pyrophosphates might operate as activators of this channel in β-cells, they clearly do not control the apparent opening of the channels under conditions allowing the "$P_i$ flush." The study also clearly demonstrates that glucose-stimulated insulin occurs in the absence of XPR1 channels, in agreement with previous findings (1).

The evidence presented above suggests that phosphate efflux via the XPR1 channel is regulated by intracellular factors closely related to the energy state of the β-cell and by its inositol pyrophosphate levels rather than by extracellular $P_i$. In the search for such factor(s), the basic design features of pancreatic β-cells, which function as largely autonomous glucose sensor cells, must be kept mind (14–16). One should consider cytosolic factors that are closely related to the energy state (e.g., ATP, P-Cr, ADP, AMP, $P_i$, pH, and sodium). These are prominently influenced in stepwise transitions from "hypoglycemic" unresponsive (or deenergized) to the "normoglycemic" near threshold (or reenergized) and finally stimulated (or supercharged) states. They might act directly on the channel or indirectly by modifying kinase or phosphatase activities. They might act alone or in combination. 5′-AMP, which drops precipitously in parallel with or perhaps even faster than $P_i$ during this transition, stands out as such a molecule (7). It operates as general cellular energy state marker and could be involved here (17). The marked increase in phosphate at depleted energy states might occur due to XPR1 inhibition/closure. The "$P_i$ flush," seen only when cellular phosphate is elevated, would then be explained by deinhibition/opening of the channels when energy state recovers. Medically important, the considerations of this article and the commentary point to intracellular inorganic phosphate of β-cells as a potential pathogenetic factor and also suggest that a critical decrease of β-cell energy state is likely to increase the level of this possibly toxic molecule, a mechanistic insight of potential therapeutic relevance.

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