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Is the glucose-induced phosphate flush in pancreatic islets attributable to gating of volume-sensitive anion channels?

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Abstract D-Glucose and other nutrient insulin secretagogues have long been known to induce a transient increase in inorganic phosphate release from pancreatic islets, a phenomenon currently referred to as a ''phosphate flush''. The objective of this study was to explore the possible participation of volume-sensitive anion channels in such a process. Rat pancreatic islets were preincubated for 60 min in the presence of [^{32}P]orthophosphate and then perifused for 90 min to measure ^{32}P fractional outflow rate and insulin secretion. From minutes 46 to 70 inclusive either the concentration of D-glucose was increased from 1.1 to 8.3 mmol L^{-1} or the extracellular osmolarity was decreased by reducing the NaCl concentration by 50 mmol L^{-1}. The increase in D-glucose concentration induced a typical phosphate flush and biphasic stimulation of insulin release. Extracellular hypoosmolarity caused a monophasic increase in both effluent radioactivity and insulin output. The inhibitor of volume-sensitive anion channels 5-nitro-2-(3-phenylpropylamino)benzoate (0.1 mmol L^{-1}) inhibited both stimulation of insulin release and phosphate flush induced by either the increase in D-glucose concentration or extracellular hypoosmolarity. It is proposed that gating of volume-sensitive anion channels accounts for the occurrence of the phosphate flush and subsequent stimulation of insulin secretion in response to either an increase in D-glucose concentration or a decrease in extracellular osmolarity.

Keywords Rat pancreatic islets · Phosphate flush · Insulin release · Volume-sensitive anion channels (VSAC)

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Introduction

D-Glucose and other nutrient secretagogues transiently increase effluent radioactivity from pancreatic islets prelabelled with [^{32}P]orthophosphate [1–3]. This coincides with a sizeable decrease in the inorganic phosphate content of the islets [4]. In this report the proposal is made that this phenomenon, currently referred to as a phosphate flush, is mainly attributable to gating of volume-sensitive anion channels (VSAC).

Results

Insulin secretion

The basal release of insulin recorded at 1.1 mmol L^{-1} D-glucose averaged, at minutes 31 and 45, respectively, 304 ± 46 and 307 ± 45 nU per islet per min (n = 9). In the presence of 0.1 mmol L^{-1} 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB), the corresponding values were 218 ± 18 and 211 ± 8 nU per islet per min (n = 4). These mean values were not significantly different from each other.

At the low concentration of D-glucose (1.1 mmol L^{-1}) reduction of extracellular osmolarity, as caused by a 50 mmol L^{-1} decrease in NaCl concentration, induced a rapid secretory response (Fig. 1). Indeed, within one

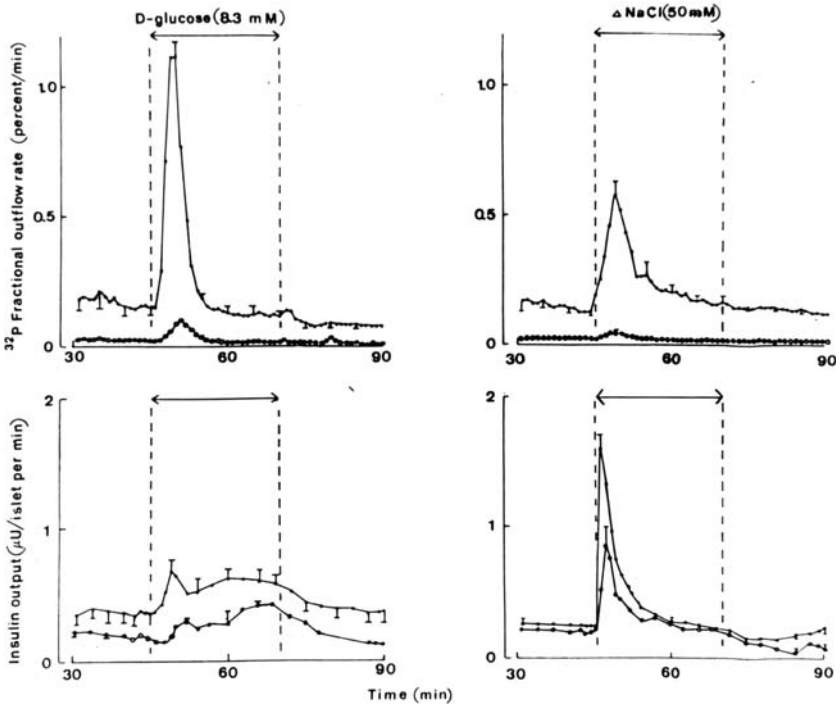

Fig. 1 Effects of an increase in D-glucose concentration from 1.1 to 8.3 mmol L^{-1} (*left*) or a decrease in extracellular osmolarity, as caused by reduction of the NaCl concentration by 50 mmol L^{-1} (*right*), between minutes 46 and 70, on ^{32}P fractional outflow rate (FOR) (*upper panels*) and insulin release (*lower panels*) from islets perifused for 90 min in the absence (*closed circles*) or presence (*open circles*) of NPPB (0.1 mmol L^{-1}). Mean values (±SEM) refer to 2–5 individual experiments

minute the output of insulin was increased by 1330 ± 99 nU per islet per min ($n = 5$; $P < 0.001$), reaching a value 6.13 ± 0.52 times higher than paired basal insulin release. The output of insulin then decreased exponentially, reaching, after exposure for 20 min to the hypotonic perifusion medium, a mean value (249 ± 21 nU per islet per min) virtually identical (paired difference –4 ± 6 nU per islet per min) with the basal insulin output recorded from minutes 44 to 45 (253 ± 26 nU per islet per min).

When comparable experiments were conducted in the presence of NPPB (0.1 mmol L^{-1}), a monophasic secretory response to extracellular hypoosmolarity was again recorded. The initial peak increment in insulin output was reduced ($P < 0.02$) to 627 ± 161 nU per islet per min ($n = 2$), however, i.e. to approximately half its control value. It was also reached one minute later in the presence of NPPB than in its absence. Under the former conditions, an exponential decrease in insulin release was also observed after the initial secretory peak, the hormonal output again reaching, after exposure for approximately 20 min to the hypotonic medium, a mean value (209 ± 25 nU per islet per min) comparable with that recorded at minute 45 (210 ± 8 nU per islet per min). The half-life for the secretory response to extracellular hypoosmolarity was also comparable in the absence of NPPB (115 ± 9 s; $n = 5$) and in its presence (107 ± 6 s; $n = 2$).

In the control experiments, conducted in the absence of NPPB, the increase in D-glucose concentration from 1.1 to 8.3 mmol L^{-1} induced a biphasic increase in insulin output. Within 4.5 ± 0.3 min, the secretory rate indeed reached a first peak value, which was 351 ± 36 nU per islet per min ($n = 4$) higher ($P < 0.005$) than that recorded at minute 45. After a further period of 4.0 ± 0.4 min exposure to the higher concentration of D-glucose, the release of insulin was 200 ± 47 nU per islet per min ($n = 4$) lower ($P < 0.025$) than the preceding peak value. The output of insulin then increased again, eventually reaching, within 7.3 ± 0.8 min, a value 123 ± 27 nU per islet per min ($n = 4$) higher ($P < 0.02$) than the preceding nadir value. At minute 75, i.e. 5 min after restoration of the low D-glucose concentration (1.1 mmol L^{-1}), the release of insulin was already 159 ± 17 nU per islet per min ($n = 4$) lower ($P < 0.005$) than the secretory rate recorded at minute 69 at the end of the period of exposure to the high concentration of D-glucose (8.3 mmol L^{-1}). Perifusion of the islets at the low sugar concentration (1.1 mmol L^{-1}) for a period of 18.0 ± 1.4 min was required, however, to reach a nadir value close to that recorded at minute 45 with a paired difference of 7 ± 21 nU per islet per min ($n = 4$).

In the presence of NPPB the increase in D-glucose concentration also stimulated insulin release in a multiphasic pattern. The presence of NPPB, however, affected the secretory response to D-glucose in two respects. First, the magnitude of both the initial increase in insulin output to reach the first secretory peak and the subsequent decrease in secretory rate to reach its nadir value was lower in the presence of NPPB than in its absence, averaging, under the former conditions, 44.1 ± 8.2 % ($n = 4$; $P < 0.02$) of the mean corresponding values recorded in the control experiments (100.0 ± 12.0 %; $n = 8$). Likewise,

over the first 9 min of exposure to 8.3 mmol L^{-1} D-glucose the increment in insulin output was significantly lower ($P < 0.02$) in the presence of NPPB (65 ± 10 nU per islet per min; $n = 2$) than in its absence (177 ± 17 nU per islet per min; $n = 4$). The mean length of exposure to 8.3 mmol L^{-1} D-glucose required to reach the first secretory peak, later nadir value, and subsequent higher insulin output was always higher in the experiments conducted in the presence of NPPB than in the control experiments conducted in its absence. The NPPB-induced delay in these three secretory events averaged 35.8 ± 9.3% (d.f. = 12; $P < 0.005$) of the corresponding mean length of exposure to 8.3 mmol L^{-1} D-glucose recorded in the control experiments.

^{32}P fractional outflow rate (FOR)

The basal ^{32}P FOR recorded at 1.1 mmol L^{-1} D-glucose averaged, at minutes 31 and 44 respectively, 0.177 ± 0.030 and 0.135 ± 0.016 × 10^{-2} min^{-1} ($n = 12$ in both cases). Over this period, a modest decrease in ^{32}P FOR was observed, the value reached at minute 44 averaging 84.1 ± 4.3% ($n = 12$; $P < 0.005$) of the paired measurement made at minute 31.

Exposure of the prelabelled islets to the hypoosmolar medium resulted in a monophasic increase in ^{32}P FOR. Already at minute 46, the ^{32}P FOR averaged 142.7 ± 7.1 % ($n = 6$; $P < 0.005$) of the paired value recorded at minute 45. It reached, 3.5 ± 0.5 min after minute 45, a peak value 0.489 ± 0.039 × 10^{-2} min^{-1} higher than the paired measurement made at minute 44. Thereafter, the ^{32}P FOR decreased exponentially. At minute 70 it was no longer significantly higher (+0.039 ± 0.018 × 10^{-2} min^{-1}; $n = 6$; $P < 0.09$) than the paired value recorded at minute 44. Between minutes 66 and 69 ^{32}P FOR continued to decrease at a mean rate of 0.007 ± 0.001 × 10^{-2} min^{-1} ($P < 0.005$). Over the ensuing 3 min (minutes 70–72), however, the regression coefficient for the decrease in ^{32}P FOR was, except in one experiment, higher, averaging 151.6 ± 18.0% ($n = 5$; $P < 0.005$) of the paired value recorded between minutes 66 and 69.

In the presence of NPPB, the basal ^{32}P FOR decreased ($P < 0.05$) from 0.026 ± 0.002 to 0.021 ± 0.001 ($n = 4$ in both cases) between minutes 31 and 44. These values were much lower ($P < 0.02$ or less) than those recorded at the same time in the control experiments conducted in the absence of NPPB.

In the presence of NPPB, exposure of the islets to the hypoosmolar medium progressively increased ($P < 0.05$ or less) the ^{32}P FOR from 0.020 ± 0.001 to 0.043 ± 0.011 × 10^{-2} min^{-1} ($n = 2$) between minutes 45 and 49. In absolute terms this increment was approximately a factor of 20 less than that recorded in the absence of NPPB. In the presence of NPPB, the ^{32}P FOR progressively decreased after minute 49. At minute 70, it averaged no more than 0.016 ± 0.002×10^{-2} min^{-1}.

The increase in D-glucose concentration from 1.1 to 8.3 mmol L^{-1} also induced, in the absence of NPPB, a monophasic increase in ^{32}P FOR. The peak value was reached 4.7 ± 0.2 min after the increase in D-glucose concentration, being 1.051 ± 0.094 × 10^{-2} min^{-1} higher ($n = 6$; $P < 0.001$) than the paired measurement made at minute 45. Such an increment was twice as high ($P < 0.001$) as that recorded in response to extracellular hypoosmolarity. At minute 56, the ^{32}P FOR was already back to basal value, the paired difference with the measurements made at minute 45 not exceeding 0.007 ± 0.016 × 10^{-2} min^{-1} ($n = 6$). Nevertheless, when the concentration of D-glucose was brought back to 1.1 mmol L^{-1}, and after a transient and modest off-response, further acceleration of the decrease in ^{32}P FOR was observed. Indeed, while the ^{32}P FOR decreased by only 0.003 ± 0.009 × 10^{-2} min^{-1} ($n = 6$; $P < 0.005$) between minutes 65 and 70, it decreased by 0.032 ± 0.006 × 10^{-2} min^{-1} ($n = 6$; $P < 0.005$) between minutes 70 and 75. The biological significance of this difference ($P < 0.025$) is reinforced by the occurrence of the above-mentioned short-lived off-response.

The anionic response to the increase in D-glucose concentration, like that evoked by extracellular hypoosmolarity, was severely impaired in the presence of NPPB. Although the ^{32}P FOR from the islets perfused in the presence of NPPB increased significantly ($P < 0.01$ or less) over the first four to five minutes of exposure to 8.3 mmol L^{-1} D-glucose, the peak value reached at minute 50.5 ± 0.5 averaged no more than 0.100 ± 0.003 × 10^{-2} min^{-1} ($n = 2$), as distinct ($P < 0.001$) from 1.1943 ± 0.076 × 10^{-2} min^{-1} ($n = 6$) in the control experiments conducted in the absence of NPPB. Thereafter, the ^{32}P FOR from the islets exposed to NPPB progressively decreased reaching, at minute 57, a value of 0.024 ± 0.003 × 10^{-2} min^{-1}, close to the basal value (minute 44) of 0.022 ± 0.062 × 10^{-2} min^{-1}. The time course of the anionic response to the rise in D-glucose concentration was thus virtually identical in the absence and presence of NPPB.

Discussion

This study confirms that an increase in D-glucose concentration induces biphasic stimulation of insulin release from rat pancreatic islets which coincides with a typical monophasic phosphate flush. These results are also in agreement with previous results indicating that extracellular

hypoosmolarity induces a rapid increase in insulin output followed by an exponential return of the secretory rate toward basal value [5–8].

Inhibition, by NPPB, of the secretory response to either D-glucose [9] or extracellular hypoosmolarity [10] has also been reported previously.

This work has, however, afforded two novel pieces of information. First, it has revealed that stimulation of insulin release by extracellular hypoosmolarity coincides with a phosphate flush comparable with, albeit less marked than, that induced by an increase in D-glucose concentration. Second, it has revealed that in islets stimulated by either a increase in D-glucose concentration or a decrease in extracellular osmolarity the inhibitor of VSAC NPPB severely opposes the increase in effluent radioactivity otherwise recorded from the islets prelabelled with [^{32}P]orthophosphate.

It is tempting, therefore, to propose that, in response to either D-glucose [11] or extracellular hypoosmolarity [7, 8, 10–12], the known increase in the volume of insulin-producing cells leads to gating of VSAC and that this accounts for the occurrence of a phosphate flush. Such a process may, in turn, lead to further depolarization of the plasma membrane and, hence, to gating of voltage-dependent Ca^{2+} channels.

In insulin release induced by D-glucose or other nutrient secretagogues, another anion or anions other than inorganic phosphate may participate in the second phase of the insulin secretory response. They may include organic anions (e.g. pyruvic and lactic acids) generated by catabolism of the nutrient secretagogues or, as suggested by our work on the participation of carbonic anhydrase in the stimulus-secretion coupling of nutrient-induced insulin release [13], the outflow of bicarbonate anions generated in the reaction catalysed by carbonic anhydrase from CO_2, itself produced in the oxidative catabolism of such nutrient secretagogues.

Materials and methods

All experiments were conducted on pancreatic islets isolated by the collagenase procedure [14] from fed female albino rats. The methods used to measure both insulin release and effluent radioactivity from islets prelabelled with [^{32}P]orthophosphate and placed in a perifusion chamber have been described elsewhere [3, 15]. Briefly, groups of 110 islets each were preincubated for 60 min at 37°C in 240 μL Hepes and bicarbonate-buffered salt-balanced medium [16] containing bovine serum albumin (5.0 mg mL^{-1}), D-glucose (16.7 mmol L^{-1}), and Na_2PO_4 (1.0 mmol L^{-1}) mixed with a trace amount of [^{32}P]orthosphophate (7.4 MBq mL^{-1}). The islets were then perifused for the first 45 min and the last 20 min with the same medium, except for the absence of labelled orthophosphate and the presence of a lower concentration of D-glucose (1.1 mmol L^{-1}). From minutes 46 to 70 inclusive, either the concentration of D-glucose was increased to 8.3 mmol L^{-1} or the extracellular osmolarity was decreased by reducing the NaCl concentration by 50 mmol L^{-1}. The islets were thus perifused for 90 min at a flow rate of 1.0 mL min^{-1}, the effluent being collected over successive periods of 1 min each and examined for both insulin content by radioimmunoassay and radioactivity content by use of the Cerenkov counting technique. The efflux of ^{32}P was expressed as an instantaneous fractional outflow rate (FOR), taking into account the accumulated values for effluent radioactivity and the final radioactive content of the islets. In some experiments the perfusate administered throughout the experiment contained 0.1 mmol L^{-1} 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB), an inhibitor of volume-sensitive anion channels. All data are expressed as mean values (±SEM) with the number of individual observations (n) or degree of freedom (d.f.). The statistical significance of differences between mean values was assessed by use of Student's t-test.

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