An Inhibitor of Sweet Taste Response Modulates Glucose-Induced Phosphoinositide Breakdown in Rat Pancreatic Islets

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Abstract. We studied the effect of a specific-competitive inhibitor of the sucrose taste response, p-nitrophenyl-D-glucopyranoside (PNP-Glu) on insulin release and phosphoinositide metabolism in rat pancreatic islets. The α -anomer, but not the β -anomer, of PNP-Glu at a concentration of 5 mM inhibited insulin release induced by 10 mM glucose. Islets were labeled by exposure for 2 h to 10 μ Ci of myo-[2- 3 H] inositol solution supplemented with 2.8 mM glucose. Forty islets were then incubated in the presence of 10 mM LiCl, 1 mM inositol and 10 mM glucose with or without the anomers of PNP-Glu. [3 H] radioactivity in the incubation medium remained significantly greater in the presence of the α -anomer of PNP-Glu than in the presence of glucose alone after 5- and 20-min incubation. The inositol monophosphate levels in the islets incubated with glucose alone were increased more than in the islets with α -anomer. The β -anomer of PNP-Glu did not change either glucose-induced insulin release or phosphoinositide breakdown. A patch-clamp study revealed that neither anomer affected the glucose-dependent ATP-sensitive K^+ -channels. These results indicate that the anomeric preference for glucose in insulin release in the pancreatic islets is closely associated with phosphoinositide breakdown.

Key words: Insulin release, Sweet taste response, Islet, Phosphoinositide breakdown.

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IT HAS been widely accepted that glucose-induced insulin release is initiated by glucose metabolism in B-cells and that the ATP-sensitive K^+ channel plays a role in controlling B-cell function [1–3]. Although phosphoinositide (PI) breakdown may play an important role in the signal-transduction mechanism in pancreatic B-cells [4, 5], it remains unclear whether glucose metabolism is directly related to PI breakdown.

Accumulating evidence suggests that common mechanisms are involved in the signal transduction in the sweet taste response of tongue gustatory cells and glucose-induced insulin secretion from pancreatic B-cells [6–8]. Both cells recognize

glucose as a physiological stimulant and prefer the α -anomer over the β -anomer of glucose. It is well established that the α -anomer of D-glucose causes greater insulin secretion than the β -anomer of D-glucose from pancreatic islets [9–11].

P-nitrophenyl-D-glucopyranoside (PNP-Glu), a specific-competitive inhibitor of the taste response to sucrose, binds to the glucopyranosyl subsite of the sucrose receptor site with α -anomeric preference [12]. Niki *et al.* recently reported that the α -anomer, but not the β -anomer, of PNP-Glu dose dependently inhibited the release of insulin, induced by 10 mM glucose, from rat pancreatic islets [13], suggesting that PNP-Glu has a possible common mode of chemosensory action in the gustatory cells and the pancreatic B-cells. The taste response can be induced by PI breakdown [14]. In the present study, we examined the effect of anomers of PNP-Glu on glucose-induced PI break-

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down in rat pancreatic islets. We also investigated whether this compound affects the ATP-sensitive K^+ channel.

Materials and Methods

By using the collagenase digestion method [15], pancreatic islets were isolated from Wistar strain male rats (Shizuoka Laboratory Center, Hamamatsu, Japan) fed a standard laboratory diet *ad libitum*. In the first series of experiments, only insulin release was examined. The islets were preincubated for 30 min in Krebs-Ringer bicarbonate buffer (KRB) containing 2.8 mM glucose and 0.25% bovine serum albumin (BSA) under 95% O_2 /5% CO_2 . Each batch of five islets was then incubated for 60 min at 37°C in medium containing various concentrations of glucose, and either the α - or β -anomer of PNP-Glu at a concentration of 5 mM.

In the second series of experiments, islets were labeled with 10 μ Ci of myo-[2- 3H] inositol (specific activity, 18.9 Ci/mmol, 1 Ci=37 GBq, Amersham, Arlington Heights, IL) in 0.2 ml of KRB containing 2.8 or 28 mmol of glucose and 0.25% BSA for 2 h under 95% O_2 /5% CO_2 at 37°C. The labeled islets were washed five times with 1 ml of KRB without radioactive inositol. Forty islets were then incubated in the presence of 10 mM glucose, 10 mM LiCl and 1 mM inositol. The anomers of PNP-Glu were also added in concentrations of 5 mM. To study the time course of inositol metabolism, incubation was terminated at 0, 5 and 20 min by the addition of 10% perchloric acid [16] and the inositol phosphates of the islets were separated out by anion exchange chromatography [17]. Samples of the eluate were then counted in a liquid

scintillation spectrometer. Insulin levels were measured by radioimmunoassay using rat insulin as a standard [18].

In the third series of experiments, a patch-clamp study was performed according to the method described by Misler *et al.* [3]. Islets were dissociated into single cells or small clusters by pipetting in the medium without Ca^{2+} . The cells were plated on petri dishes and maintained for 3–4 days in 2 ml of the MEM tissue culture medium (Flow Laboratories, Irvine, UK), which was supplemented with 5.6 mM glucose, 10% heat-inactivated fetal bovine serum (Flow), 100 IU of penicillin per ml and 100 μ g of streptomycin per ml. The bathing solution contained 135 mM NaCl, 5 mM $MgSO_4 \cdot 7H_2O$, 5 mM $CaCl_2$, 5 mM KCl and 5 mM HEPES adjusted with KOH to pH 7.4. The solution used to fill the patch pipette consisted of 140 mM KCl, 1.13 mM $MgCl_2$, 0.1 mM $CaCl_2$ and 10 mM HEPES adjusted with KOH to pH 7.4. The anomers of PNP-Glu, LiCl and inositol were purchased from Sigma Chemical Co. (St. Louis, MO). The ^{125}I -labeled insulin was purchased from Dainabot Co. (Tokyo, Japan) and the rat insulin was a gift from Novo (Bagsvaerd, Denmark).

Values represent the mean $\pm$ SEM. Statistical analysis was performed by unpaired Student's *t*-test. For comparing more than three means, Scheffe's method was used. A *P* value below 0.05 was considered statistically significant.

Results

The basal release of insulin from pancreatic islets in 2.8 mM glucose was not affected by either the α - or β -anomer of PNP-Glu at concentrations

Table 1. Effect of the anomers of PNP-Glu on insulin release from rat islets

Glucose concentration (mM)	Control	Insulin release (ng/islet/h)	
		PNP- α -Glu	PNP- β -Glu
2.8	0.4 $\pm$ 0.0 (10)	0.3 $\pm$ 0.0 (10)	0.4 $\pm$ 0.1 (10)
10.0	4.9 $\pm$ 0.6 (14)	3.8 $\pm$ 0.5 (12)*	5.0 $\pm$ 0.5 (12)
16.7	14.6 $\pm$ 3.1 (13)	15.5 $\pm$ 3.4 (7)	12.8 $\pm$ 1.8 (8)

Islets isolated from rat pancreas were incubated for 30 min in KRB containing 2.8 mM glucose. Batches of five islets were then incubated for 1 h in either the presence or absence of the anomers of PNP-Glu. Mean values ($\pm$ SEM) for insulin release are shown. The numbers of individual observations are indicated in parentheses. *, *P*<0.05 *vs.* 10 mM glucose alone and plus PNP- β -Glu.

of 5 mM (Table 1). Insulin release in response to 10 mM glucose was significantly reduced by the α -anomer of PNP-Glu. The β -anomer of PNP-Glu itself did not affect insulin release caused by 10 mM glucose. When the glucose concentration in the medium was raised to 16.7 mM, insulin release was not affected by the addition of the anomers of PNP-Glu.

The islets were labeled with [^3H] inositol in the presence of 2.8 or 28.0 mM glucose. When the islets were labeled with [^3H] inositol in the presence of 28.0 mM glucose, [^3H] inositol incorporation into the islets was significantly decreased (Table 2).

As shown in Table 3, [^3H] radioactivity in the incubation medium, i.e., [^3H] efflux, was increased to a greater extent in the islets exposed to the α -anomer than in the islets exposed to the β -

anomer or glucose alone. When the incubation period was prolonged to 20 min, [^3H] efflux further increased in the islets and the difference between the islets incubated with the α - or β -anomer of PNP-Glu became more apparent. Inositol monophosphate (IP_1) and inositol bisphosphate (IP_2) levels were lower in the islets incubated for 20 min in the presence of α -anomer than in the presence of β -anomer or glucose alone. Inositol trisphosphate (IP_3) was increased at 5 min and decreased at 20 min, but there was no statistically significant difference among the three groups. The addition of the β -anomer of PNP-Glu had no significant effect on inositol phosphate levels and insulin secretion compared with 10 mM glucose alone.

The inositol phosphate levels were markedly decreased in the islets labeled in the medium containing 28.0 mM glucose (Table 4). In contrast, the mode of action of PNP-Glu was not changed by the concentrations of glucose used in the labeling medium.

Figure 1 illustrates the effect of the α -anomer of PNP-Glu on the glucose-sensitive K^+ channel in cell-attached patches. The addition of 10 mM glucose closed the K^+ channels within 4–5 min. The α -anomer of PNP-Glu did not affect the glucose-induced closure of the K^+ channel.

Discussion

The central mechanisms by which glucose

Table 2. Influence of labeling conditions on tissue partition of [^3H] inositol in isolated islets

Labeling condition	W.S.	Radioactivity (cpm/40 islets)		T.C.
		Islet residue		
2.8 mM glucose	8125 $\pm$ 945	11423 $\pm$ 1250	19549 $\pm$ 2111	
28.0 mM glucose	2236 $\pm$ 215*	3221 $\pm$ 353*	5537 $\pm$ 509*	

Islets were incubated for 2 h with [^3H] inositol in the presence of either 2.8 or 28.0 mM glucose. They were washed five times in the same buffer without radioactive inositol, and then islets were immediately extracted with HClO_4 . Data represent the mean ($\pm$ SEM) values derived from five separate experiments. W.S., water-soluble product; T.C., total count. *, $P < 0.01$ vs. 2.8 mM glucose.

Table 3. Effects of the anomers of PNP-Glu on [^3H] efflux and [^3H] inositol phosphate levels

Condition	Time (min)	Radioactivity (cpm/40 islets)				Insulin release (ng/islet)
		Efflux	IP_1	IP_2	IP_3	
t_0	0	0	1453 $\pm$ 177	622 $\pm$ 187	316 $\pm$ 66	0
10 mM glucose						
+None	5	325 $\pm$ 49	2972 $\pm$ 556	678 $\pm$ 90	404 $\pm$ 58	0.4 $\pm$ 0.1
	20	980 $\pm$ 89	6556 $\pm$ 981	625 $\pm$ 78	281 $\pm$ 40	1.8 $\pm$ 0.2
+PNP- α -Glu	5	568 $\pm$ 25*	2054 $\pm$ 276	581 $\pm$ 36	431 $\pm$ 60	0.2 $\pm$ 0.0*
	20	1318 $\pm$ 68*	4059 $\pm$ 491*	369 $\pm$ 27	239 $\pm$ 16	1.2 $\pm$ 0.1*
+PNP- β -Glu	5	386 $\pm$ 42	2884 $\pm$ 445	705 $\pm$ 93	458 $\pm$ 47	0.4 $\pm$ 0.0
	20	977 $\pm$ 74	6387 $\pm$ 794	666 $\pm$ 58	276 $\pm$ 35	1.7 $\pm$ 0.2

After being labeled for 2 h with [^3H] inositol, the islets were washed five times with KRB containing 2.8 mM glucose and the reaction was stopped either immediately (t_0) or after an additional 5 or 20 min in either the presence or absence of the anomers of PNP-Glu. Data represent the mean ($\pm$ SEM) values derived from five separate experiments. [^3H] radioactivity in the incubation medium is defined as efflux. IP_1 , inositol monophosphate; IP_2 , inositol bisphosphate; IP_3 , inositol trisphosphate. *, $P < 0.05$ vs. 10 mM glucose alone and plus PNP- β -Glu.

Table 4. Effect of the anomers of PNP-Glu on [^3H] efflux and [^3H] inositol phosphate levels from islets labeled in 28.0 mM glucose

Condition	Time (min)	Radioactivity (cpm/40 islets)			
		Efflux	IP ₁	IP ₂	IP ₃
t_0	0	0	384 $\pm$ 53	232 $\pm$ 41	167 $\pm$ 13
10 mM glucose					
+PNP- α -Glu	5	256 $\pm$ 42	356 $\pm$ 60	189 $\pm$ 11*	141 $\pm$ 19
	20	545 $\pm$ 125	994 $\pm$ 61*	219 $\pm$ 36	170 $\pm$ 25
+PNP- β -Glu	5	203 $\pm$ 18	308 $\pm$ 45	123 $\pm$ 12	115 $\pm$ 3
	20	381 $\pm$ 39	1394 $\pm$ 43	235 $\pm$ 18	140 $\pm$ 20

Islets were incubated for 2 h with [^3H] inositol in the presence of 28.0 mM glucose. They were washed five times in the same buffer without radioactive inositol. The reaction was stopped either immediately or after an additional 5 or 20 min in the presence of 10 mM glucose and the anomers of PNP-Glu. Data represent the mean ($\pm$ SE) values derived from four separate experiments. *, $P < 0.05$ vs. PNP- β -Glu.

Fig. 1. Effect of the α -anomer of PNP-Glu on glucose-dependent K^+ channels in the B-cell. A: channel activity recorded from a cell-attached patch at a pipette potential (V_p) of +40 mV in the absence of glucose. B: Continuation of the record 7 min after adding 10 mM glucose. C: 10 min after washing with buffer alone. D: 7 min after adding 10 mM glucose plus 5 mM α -anomer of PNP-Glu. E: 10 min after washing with buffer alone.

stimulates insulin release still remain to be elucidated. The initial and key event in insulin release from pancreatic B-cells appears to be coupled with PI breakdown [4, 5]. Previous studies [19, 20] demonstrated that glucose failed to cause PI breakdown in permeabilized rat islets. However, Wolf *et al.* [21] found that glucose per se activated phospholipase C independently from glucose metabolism in permeabilized rat islets, providing direct evidence for the presence of a glucoreceptor in pancreatic B-cells. Although there are clear differences between those studies, the present study may provide additional support for the latter case.

The release of insulin evoked by 10 mM glucose was inhibited significantly by the α -anomer of PNP-Glu. This phenomenon does not result from the inhibition of glucose oxidation in the B-cells [10], indicating that glucose transport and glycolysis are not grossly impaired by PNP- α -Glu. It is noted that the anomers of PNP-Glu did not affect insulin release induced by basal and maximal concentrations of glucose. Thus, the inhibitory effect of the α -anomer of PNP-Glu on insulin release might not be due to toxic and nonspecific actions, and the effect was overcome when the glucose concentration was raised to the maximum.

To examine the possibility that the anomers of PNP-Glu interfere with glucose metabolism, we performed a patch-clamp study using a single B-cell. It has been suggested that the ATP-sensitive K^+ channel is dependent on the status of glucose metabolism, since the addition of mannoheptulose, an inhibitor of hexokinase, reopened K^+ channels closed down by glucose [1, 3]. Our finding that the α -anomer of PNP-Glu did not affect K^+ -channel activities suggests that the inhibition of glucose-induced insulin secretion by the α -anomer of PNP-Glu is independent of glucose metabolism and the subsequent ATP production in the pancreatic B-cells.

Since Li^+ inhibits the dephosphorylation of several inositol phosphates generated during stimulated PI breakdown, LiCl is often used to

increase the sensitivity of detecting inositol cycling in pancreatic islets [19, 20, 22] as well as other tissues [17]. One can expect an increase in IP_1 levels and a decrease in $[^3H]$ efflux in the presence of LiCl. However, when the α -anomer of PNP-Glu was present in the incubation medium, IP_1 levels were unexpectedly lower than in the presence of glucose alone or the β -anomer of PNP-Glu. On the other hand, $[^3H]$ efflux was markedly higher in the presence of the α -anomer than glucose alone or the β -anomer. These results strongly suggest that the α -anomer of PNP-Glu interferes with PI breakdown caused by glucose.

Rana *et al.* [23] reported that there are glucose-responsive and -unresponsive phospholipid pools in rat pancreatic islets. As shown in Table 2, high concentrations of glucose in the labeling medium somewhat inhibited the incorporation of $[^3H]$ inositol into the islet-inositol pool. This finding is consistent with the previous observation by Zawulich *et al.* [24]. Thus, when islets are labeled with radioactive inositol, high concentrations of glucose should not be used. However, even in such conditions, the data on PI breakdown yielded by the present study are quite comparable. Although our study did not establish a direct link between the action of PNP-Glu and PI breakdown, the present data suggest that anomeric preference of glucose in insulin release is closely associated with PI metabolism in pancreatic B-cells.

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