Receptors of Paraneurons, with Special Reference to Glucoreceptors

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Summary. Among glucose-recognizing paraneurons, the gustatory cell is believed to have a glucoreceptor, while the pancreatic B cell is thought to metabolize glucose for signal production for insulin release. To investigate whether a common mechanism of glucose recognition exists among these cells, we have studied the interaction of the anomers of glucose or its derivative, known as a sugar taste inhibitor, with the pancreatic B cell of normal and diabetic rats. Inhibitors of the sugar taste response inhibited glucose-induced insulin release in various manners. A non-specific inhibitor, dibucaine, impaired not only the insulin response to glucose but also the ability to discriminate the anomers of glucose, without inhibiting glucose oxidation in the islets. Dibucaine also inhibited insulin release induced by a non-glucose secretagogue, tolbutamide. The α anomer, but not the β anomer, of p-nitrophenyl-D-glucopyranoside, a specific-competitive inhibitor of the sugar taste response, inhibited glucose-induced insulin release, but did not inhibit insulin release induced by non-glucose secretagogues or glucose oxidation. The pancreatic B cell of a non-insulin-dependent diabetes (NIDD) rat model, the NSZ rat, exhibited low insulin response to glucose and did not discriminate between the two anomers of glucose. The diabetic B cell responded to non-glucose secretagogues to the same extent as the control. Glucose oxidation in the diabetic islets was not impaired. These findings, together with previous ones, suggest that the gustatory and pancreatic B cells have a common glucose recognition site, which has a steric preference for the α anomer and is impaired in NIDD.

Several paraneuron cells recognize glucose as signals for their physiological responses (FUJITA et al., 1988). These paraneuron cells include the gustatory sweet receptor cells, the pancreatic A, B and D cells, and the enteroglucagon cells in the small intestine. Among them, the pancreatic B cell and the gustatory cell have been most intensively studied, and are known to share the following common properties in their stimulus-secretion coupling (see NIKI and NIKI, 1980). First, the α anomer of glucose or mannose is more potent than the β anomer in inducing physiological responses in the two types of cells. Second, treatment of these cells with alloxan inhibits their responses to glucose, though the responses to other stimuli are preserved. Both of the cells are protected from the alloxan toxicity by the co-existence of glucose. Third, treatment of the two types of cells with some proteolytic enzymes attenuates their responses to glucose without affecting responses to other stimuli (HJII, 1975; KRAUSE et al., 1973). Fourth, proteins which bind to glucose have been reported to be extracted from the membrane fractions of the two cell types, although the exact nature of the proteins has not been determined (DASTOLI and PRICE, 1966; PRICE, 1973). Fifth, in non-insulin-dependent diabetics (NIDD), the physiological responses to glucose of the two types of cells are believed to decrease, even when they respond normally to other stimuli.

However, the proposed hypotheses on the mechanism of glucose recognition by the two types of cells differ. The gustatory cell is considered to recognize glucose at a receptor site on the cell membrane (SATO, 1985; JAKINOVICH and SUGARMAN, 1988), while in the pancreatic B cell, the metabolism of glucose is believed to initiate insulin release (HEDESKOV, 1980; ASHCROFT, 1980; MALAISSE et al., 1984). The latter concept is mainly based on the fact that insulinotropic actions of sugars are tightly linked to the rate of islet metabolism.

We here report on our recent observations on the interaction of the anomers of glucose or its derivative, known as a sugar taste inhibitor, with the pancreatic B cells of normal and diabetic rats. The findings provide further evidence to suggest a common glucoreceptor mechanism in the two types of cells.
EFFECTS OF SUGAR TASTE INHIBITORS ON GLUCOSE-INDUCED INSULIN RELEASE

The various inhibitors of the sugar taste response have been divided into three types from a mechanistic point of view; namely non-specific, specific-non-competitive, and specific-competitive (JAKINOVICH and SUGARMAN, 1988).

Local anesthetics are known to be non-specific taste inhibitors. We have studied the effects of dibucaine on glucose-induced insulin release. Perfusion of the isolated pancreas was performed according to the method previously reported (NIKI et al., 1988). Isolated pancreata were preperfused with 0.25 mM dibucaine for 5 min, and then 10 mM of the α or β anomer of glucose was infused for 20 min on the same background concentration of dibucaine. In non-treated pancreata, as shown in the left panel in Figure 1, the α anomer of glucose was significantly more potent than the β anomer in stimulating insulin release. Treatment of pancreata with 0.25 mM dibucaine reduced not only the amount of insulin release but also the ability to discriminate the α and β anomers of glucose, as shown in the right panel in Figure 1. Suppression of insulin release by dibucaine was predominantly observed in the first phase of the release induced by glucose. The inhibitory effect of dibucaine did not result from its inhibition of glucose oxidation in islets. The amount of glucose oxidized to CO₂, measured as previously described (NIKI et al., 1981), did not significantly differ between islets incubated for 30 min with 10 mM glucose alone and those with 10 mM glucose plus 0.25 mM dibucaine, 9.47±0.88 and 9.33±0.98 pmol/islet, respectively. Dibucaine also inhibited insulin release induced by a non-glucose insulin secretagogue, tolbutamide (data unshown), indicating that inhibition by the compound is not specific for glucose.

The effect of a specific-non-competitive inhibitor, gymnemic acid, on glucose-induced insulin release was inconstant, probably because of its saponin-like activity or species difference in the inhibitory effect of the compound.

Among the specific-competitive inhibitors of the sugar taste response, we have tested the effect of p-nitrophenyl-D-glucopyranoside (PNP-Glu) on glucose-induced insulin release from isolated rat pancreatic islets (NIKI et al., 1989a). PNP-Glu exists in the α or β anomeric configuration and, particularly interesting, the α anomer was reported to be more effective than the β anomer in suppressing the sugar taste response (VLAHOPoulos and JAKINOVICH, 1986). As shown in Figure 2, the α anomer of PNP-Glu, at a concentration of 5 mM, inhibited insulin release induced by 10 mM glucose by approximately 75%, while the β anomer did not significantly inhibit the release. The inhibition by the α anomer was surmounted by a higher concentration, 20 mM, of glucose (Fig. 2, the right half). The α anomer of PNP-Glu neither affected glucose oxidation in islets nor inhib-
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The inhibitory effect of PNP-Glu on insulin response to glucose accords with that observed in the gerbil's taste response to sucrose (Vlahopoulos and Jakinovich, 1986) in the following three aspects. First, the \( \alpha \) anomer of PNP-Glu was more potent than the \( \beta \) anomer in inhibiting the physiological responses of the two types of cells. Second, the inhibitory effects were surmounted by a higher concentration of sugars. Third, PNP-Glu did not affect the responses of the cells to non-sugar stimulants. These findings suggest that PNP-Glu inhibits glucose-induced insulin release in a specific, competitive manner, and via a mechanism similar to that proposed by Vlahopoulos and Jakinovich (1986) in their study on sucrose taste response; i.e., PNP-Glu binds to the glucopyranosyl subsite of the sucrose receptor site with a steric preference for the \( \alpha \) anomer. However, accumulated data on glucose recognition by the pancreatic B cell had been supportive of the hypothesis that glucose metabolism initiated insulin release, although the identity of the coupling factor(s) remained unknown. There had been no biochemical evidence which substantiated the presence of a glucoreceptor, until Wolf et al. (1988) recently reported that glucose per se stimulated phospholipase C activity independently of the glucose metabolism in digitonin-permeabilized islets. The finding suggests that the pancreatic B cell has a recognition site for glucose itself, but the nature of the site remains to be clarified. Our present findings using the anomers of PNP-Glu provide evidence that there are some common features between the glucose recognition sites of the gustatory sweet receptor cell and the pancreatic B cell.

**DISCRIMINATION OF D-GLUCOSE ANOMERS BY NIDD RAT PANCREATIC B CELL**

The similarity in the glucose recognition of the two types of cells is of great interest from a clinical point of view. The pancreatic B cell of NIDD is known to be less sensitive to glucose, even though insulin responses to non-glucose secretagogues are preserved. In addition, it was reported that the sensitivity of the sugar taste receptor was low in NIDD, despite normal sensitivity in salt detection (Lawson et al., 1979).

We have investigated whether the diabetic B cell discriminates the \( \alpha \) and \( \beta \) anomers of glucose by using a rat model of NIDD, the NSZ rat, which was induced by streptozotocin injection at 2 days of age (Niki et al., 1988, 1989b). Fasting blood glucose levels of the NSZ rats were within a normal range, but the mean maximal level after the glucose load (2 g/kg body weight) reached approximately 300 mg/dl. Figure 3 shows the effect of glucose anomers on insulin release from perfused pancreata isolated from the NSZ and control rat. In the control pancreata, 10 mM

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**Fig. 2.** Effects of the \( \alpha \) and \( \beta \) anomers of PNP-Glu on glucose-induced insulin release. Batches of 5 islets isolated from the rat pancreas were incubated for 60 min in media containing 10 mM glucose and either the \( \alpha \) or \( \beta \) anomer of PNP-Glu at a concentration of 5 mM (the left panel), and in media containing 20 mM glucose with or without the \( \alpha \) anomer of PNP-Glu at 5 mM (the right panel). Each column represents the mean (± SEM) insulin release (n=6-11).
α-glucose was significantly more potent than β-glucose both in the first and second phases. In the NSZ rat pancreata, on the other hand, insulin responses to the α and β anomers of glucose at 10 mM were not significantly different. The inability of the pancreatic B cell of NSZ rats to discriminate the two anomers is considered to result mainly from a lowered sensitivity to α-glucose, since insulin response to α-glucose was remarkably reduced while that to β-glucose was only slightly diminished. The NSZ rat pancreata responded to a non-glucose secretagogue, tolbutamide, to the same extent as the control pancreata (NIKI et al., 1989b). Glucose oxidation in the NSZ rat islets was not significantly different from that in the control rat islets (NIKI et al., 1989b).

For the NSZ rat, there is good agreement among reports on the selective impairment of insulin response to glucose (WEIR et al., 1986). Insulin responses to non-glucose secretagogues have been reported to be normal or even higher than those in controls (GIROIX et al., 1983; WEIR et al., 1986). The findings suggest that the impairment of the diabetic B cell is at the step of glucose recognition, rather than in the common exocytotic processes. Whether or not the selective defect of glucose recognition results from an impaired glucose metabolism in the islets remains unsettled. That the rate of glucose utilization in the NSZ rat islet is not lowered has been reported by COLELLA et al. (1987) and PORTHA et al. (1988), suggesting that the glycolytic flux is not altered in the diabetic islet. The results of glucose oxidation in the NSZ rat islet have not been consistent. Our present findings as well as the findings reported by OSTENSON (1985) show that the rate of glucose oxidation in the NSZ rat islet is comparable to that in the control islet, while PORTHA et al. (1988) recently reported reduced rates of oxygen uptake and glucose oxidation. Thus, they assumed that the defect in the diabetic islet reflected an alteration in mitochondrial function.

The pancreatic B cell of the NSZ rat exhibits not only low insulin response to equilibrated glucose but also a defective discrimination between the α and β anomers of the sugar. This finding suggests that the pancreatic B cell function which is impaired in the NSZ rat is related to the mechanism by which the B cell discriminates the two anomers. Although the mechanism is still a matter of debate, it has been proposed that the pancreatic B cell discriminates the two anomers in glycolytic enzyme(s) with the spe-

![Fig. 3](image-url)  
**Fig. 3.** Effects of the α (●——●) and β (○——○) anomers of glucose on insulin release from the isolated perfused pancreas of control (a) and NSZ rats (b). After 20-min preperfusion with 1.7 mM equilibrated glucose, α- or β-glucose at a concentration of 10 mM was introduced for 20 min. Values represent mean (±SEM) insulin levels (n=5). (NIKI et al., 1988).
cificity for the α anomer, namely in glucokinase (MEGLASSON and MATSCHINSKY, 1984), phosphogluco-
se isomerase and/or phosphoglucomutase (MALAISSE and SENER, 1985). If one of these glycolytic enzymes
is impaired, it should result in a decrease in glucose utilization; such is not the case in the NSZ rat islet.
Glycolytic products distal to triose-phosphate do not exist in anomeric configurations. Thus, mitochondrial
oxidation can not be the step where the pancreatic B cell discriminates the two anomers.

Defective discrimination of the two anomers of glucose by the pancreatic B cell has been observed
not only in the NSZ rat but also in the BB rat, a model of insulin-dependent diabetes (LECLERCQ-
MEYER et al., 1987). We have recently observed the defective function in another NIDD rat model, the
GK rat, which was produced by the selective breeding of normal rats (GOTO et al., 1975). All these rat models
differ in their causes and types of diabetes; a common abnormality may be hyperglycemia. WEIR et al. (1986)
proposed a hypothesis that chronic hyperglycemia results in desensitization of the pancreatic B cell to
sugar, a phenomenon analogous to well-known receptor desensitization (FISHMAN and PERKINS,
1988).

REMAINING PROBLEMS

There is no doubt that sweet taste response can be induced independently of the metabolism of stimuli,
since the stimuli include non-metabolizable substances or substances which do not penetrate the cell
membrane. However, it has not been precisely determined whether glucose induces the sweet taste
response via a common receptor(s) which recognizes the above mentioned stimuli. No receptor protein(s)
for sweet stimuli has ever been identified, either. On the other hand, insulin release induced by sugars
parallels, without any exception so far, their metabolism in pancreatic islets. An interpretation of the
parallelism between sugar recognition by the gustatory and pancreatic B cells, as described in this
communication, also needs caution; common features may merely be a reflex of common properties of a
receptor protein and enzymes responsible for the sugar metabolism. All these considerations remain to
be clarified in the future.

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REFERENCES

ASHCROFT, S. J. H.: Glucoreceptor mechanisms and the control of insulin release and biosynthesis. Diabetologia

COLELLA, R. M., J. M. MAY, S. BONNER-WIEIR, J. L. LEA-
HY and G. C. WEIR: Glucose utilization in islets of hyperglycemic rat models with impaired glucose-in-


FISHMAN, P. H. and J. P. PERKINS: Receptor desensit-
ization. In: (ed. by) R. ADELSTEIN, C. KLEE and M.
RODBELL: Advances in second messenger and phospho-
(p. 25-32).

FUJITA, T., T. KANNO and S. KOBAYASHI: The para-

GIROIX, M.-H., B. PORTHA, M. KERGOAT, D. BAILBE and L.
PICON: Glucose insensitivity and amino-acid hyper-
sensitivity of insulin release in rats with non-insulin-

GOTO, Y., M. KAKIZAKI and N. MASAKI: Spontaneous
diabetes produced by selective breeding of normal

HEDESKOV, C. J.: Mechanism of glucose-induced insulin

HIU, Y.: Selective elimination of taste responses to su-
(1975).

JAKINOVICH, W., Jr. and D. SUGARMAN: Sugar taste

KRAUSE, U., H. PUCHINGER and A. WACKER: Inhibition of glucose-induced insulin secretion in trypsin-treated
islets of Langerhans. Horm. Metab. Res. 5: 325-329
(1975).

LAWSON, W. B., A. ZEIDLER and A. RUBENSTEIN: Taste
detection and preferences in diabetics and their rela-

LECLERCQ-MEYER, V., J. MARCHAND and W. J. MALAISSE:
Alteration of the insulin secretory response to D-
glucose anomers in diabetic BB rats. Med. Sci. Res. 15:
1535-1536 (1987).

MALAISSE, W. J. and A. SENER: Glucokinase is not the
pancreatic B-cell glucoreceptor. Diabetologia 28: 520-
527 (1985).


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