Distribution of catecholaminergic receptors in the rat’s pancreas islet

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Summary

The effects of noradrenaline, adrenaline, isoproterenol, dopamine, apomorphine and their blockers on biphasic insulin secretion induced by 0.3% glucose were observed by means of a modified Lacy’s perifusion method.

Noradrenaline and adrenaline completely inhibit biphasic insulin secretion, and their effects disappeared completely after pretreatment with phentolamine. Dopamine inhibited the first phase completely and inhibited the second phase partially, and the inhibitory action of the dopamine disappeared after the pretreatment with phentolamine or propranolol. Isoproterenol had no effect on glucose-induced insulin secretion, whereas after treatment with phentolamine, isoproterenol produced a first-phase type of response. Domperidone blocked the effect of dopamine on the suppression of the first phase response. Apomorphine produced the second phase suppression slightly. It was concluded that the sympathetic α-receptor and DA1 was distributed on the B-cells, the sympathetic β2-receptors on the D-cell and the DA2 on the varicosity of the sympathetic β2 neuron.

Key words: perifused pancreatic islet, catecholaminergic receptor, dopamine receptor, domperidone, apomorphine

Introduction

Catecholaminergic neurons innervate and have a powerful influence on the functions of the pancreas islets1-9). Especially, administration of noradrenaline (NA) or adrenaline (Ad) suppressed insulin release from the B-cells10-12). But it has not been determined whether these sympathetic chemical transmitters act directly, since the insulin turn-over rate (in–out) across the B-cell membrane is considered to be negatively controlled by the information from the somatostatin-relay14-18). If a chemical transmitter acts stimulatively on the D-cells, the insulin-release from the B-cells would be decreased. Separative aspects of the functions of the B-cell and the D-cell will be discussed only when the data obtained can be analytically examined. This perifusion method has enabled us to analyse the immunoreactive insulin (IRI)-increasing curve after glucose application. The present communication shows that some catecholamines cause various changes in the configurations of the glucose-induced IRI-increasing curves. It also discusses the distribution of the catecholaminergic receptors in the islets.
Materials and Methods

Male Donryu rats weighing 250-300 g were used. Rat pancreas islets were isolated using the collagenase method\(^{19}\) and perifused using a modification of Lacy's method\(^{20}\).

1. Collagenase method of islet isolation

After injection of pilocarpine hydrochloride (10 mg/body i.p. Wako Pure Chemical Co.)\(^{21,22}\), the animals were anesthetized with sodium pentobarbital (25 mg/kg i.p. Dainippon Pharmaceutical Co.). Twenty ml of Hanks' solution was injected through the pancreatic duct in order to produce edema in the pancreas tissue. Then the pancreas was placed in cold Hanks’ solution and cut into small pieces with a blade on a silicon plate. Digestion of the pancreas tissue was performed with collagenase (2000 U, Cooper Biomedical Co.) and 10% fetal calf serum (Gibco Lab.) at 37°C for 15 min, using a magnetic stirrer at 400 rpm. After digestion, the islet, measuring 150-200 \(\mu m\) in diameter, was transferred into fresh cold Hanks’ solution.

2. Perfusion apparatus and procedures

A glass tube of 1 mm inner diameter and 20 mm in length was prepared. A 0.3 mg of glass wool (Nihon Sekieigarasu Co.) was inserted as a filter into the glass tube of which the central part was narrowed to 0.7-0.8 mm, and then twenty islets were placed on the glass wool. Both ends of the glass tube were joined with polyethylene tubes with an interdiameter of 1 mm. One side of the polyethylene tube was connected to a peristaltic pump (Atto Co.), the other to a fraction collector. This perfusion apparatus was placed in a 37°C water bath. Hanks' solution containing 10% fetal calf serum and 0.1% glucose was used for the perfusion medium, and the flow rate was maintained at 1 ml/10 min by means of a peristaltic pump. The total volume of this perfusion apparatus was set at 0.15 ml. The test drugs were added to the perfusion medium, and IRI in the out-flow was measured by radioimmunoassay using insulin riabead (Dainabot).

The drugs tested in the present experiment were always added into the perfusate. The compounds used were as following; dl-norepinephrine (Sankyo Co.), epinephrine (Daiichi Pharmaceutical Co.), \(\alpha\)-isoproterenol hydrochloride (Nikken Chemicals Co.), phentolamine mesylate (Ciba-Geigy), propranolol hydrochloride (ICI Pharmaceutical Co.), dopamine hydrochloride (Nakarai Chemicals Co.), apomorphine hydrochloride (Sigma Chemical Co.), domperidone, 5-chloro-1-[[3-(2,3-dihydro-2-oxo-1H-benzimidazol-1-yl) propyl]-4-piperidinyl] 1,3-dihydro-2H-benzimidazol-2-one (Kyowa Hakko Kogyo Co.) and synthetic somatostatin (Sigma Chemical Co.).

Results

Changes in the out-flow IRI levels after perfusion with 0.3% glucose containing Hanks’ solution are illustrated in Fig. 1. After the beginning of high glucose infusion, the mean curve for 17 cases showed a sudden rise for 3 min and then, after transitory falling the curve rose and stabilized at a higher level. The former is designated as the 1st phase response and the latter as the 2nd phase response.

During perfusion with either 0.1-3 \(\mu g/ml\) NA or Ad, the 1st and the 2nd phase responses totally disappeared. The suppression-mode of a small amount of NA or Ad (0.1 \(\mu g/ml\)) was similar to that of a relatively large amount of NA or Ad (3 \(\mu g/ml\)), and it was observed equivalent inhibitions on both the 1st phase and the 2nd phase response even if the concentration decreased.
Fig. 1 Increasing curve of IRI concentration is detected by pancreas islets perifusion method. Initially, 0.1% of glucose was applied, and later 0.3% of glucose was added. Each point represents the mean of 17 trials, and vertical bars indicate the standard error.

Therefore the sympathetic $\alpha$-systems are thought to be distributed homogeneously in the islets. Furthermore during perifusion with 10 $\mu$g/ml of phentolamine, neither NA nor Ad inhibited the glucose-induced IRI increase (Fig. 2).

The action-mode for isoproterenol was complicated. Although 1-3 $\mu$g/ml of isoproterenol did not have any effect on the glucose stimulated IRI increase, 1-3 $\mu$g/ml of isoproterenol accompanied with 10 $\mu$g/ml of phentolamine produced an immediate transitory rise and gradually lowered the level of IRI concentration in the out-flow thereafter (Fig. 3).

Fig. 4 illustrates the influence of 3 $\mu$g/ml of dopamine. This dose of dopamine totally suppressed the 1st phase response and partially depressed the 2nd phase one. A relatively small amount of dopamine (1 $\mu$g/ml) completely suppressed the 1st phase response without having any effect on the 2nd phase response. As shown in Fig. 4, the action-mode of dopamine resembled that of somatostatin, since small doses of somatostatin produced a total suppression of the 1st phase response and a partial suppression of the 2nd phase response. The treatment, however, with 10 $\mu$g/ml of phentolamine blocked the actions of dopamine and somatostatin, whereas the treatment
Fig. 3 When the perfusion medium contains phentolamine, the IRI-increasing response after isoproterenol administration resembles the 1st phase response. Values are the means of five preparations, S.E. is shown by vertical lines.

Fig. 4 Dopamine inhibits the 1st phase totally and the 2nd phase partially. These inhibitions disappear following pretreatment with phentolamine or propranolol. Values are the means of five preparations, S.E. is shown by vertical lines.

with 10 μg/ml of propranolol inhibited the action of dopamine only (Fig. 4 and 5).

A 0.3-1 μg/ml dose of domperidone did not affect the glucose-induced IRI-release. But, the treatment with this compound blocked the dopamine-induced suppression of the 1st phase response (Fig. 6). On the other hand, the same treatment did not inhibit the actions of NA or Ad.

Partial suppression of the 2nd phase response was always caused by 10-30 μg/ml of apomorphine while the 1st phase response remained even after the treatment with 30 μg/ml of apomorphine.

Discussion

The distribution of the catecholaminergic receptors in the pancreas islets was recently
Fig. 5 Somatostatin inhibits the 1st phase response selectively without producing any change in the 2nd phase. This action of somatostatin on the 1st phase response disappears after the pretreatment with phentolamine. But propranolol has no effect on this action. Values are the means of five preparations, S.E. is shown by vertical lines.

Fig. 6 Domperidone blocks the 1st phase suppressing effect induced by dopamine. Apomorphine induces the 2nd phase suppression slightly. Values are the means of five preparations, S.E. is shown by vertical lines.

studied by Smith et al.\textsuperscript{23} and Itoh et al.\textsuperscript{24} They both described the results obtained from isolated rat's pancreas perfusion experiments, where the rise and fall of concentrations of glucagon, insulin and somatostatin in the perfusate were used as indices for the functions of the A-cells, B-cells and D-cells, respectively. Their results, however, did not explain the mode of innervation by the autonomic neurons in the islet. The modified pancreas islet perfusion method in this
report was very useful for the observation of the functions of the B-cells and the D-cells.

In the present experiment, NA and Ad suppressed both the 1st and the 2nd phase response, and during the treatment with phentolamine, the effects of NA or Ad described above disappeared. Consequently, the sympathetic $\alpha$-receptors were thought to be richly distributed on the B-cells and the D-cells, while none of the sympathetic $\beta_1$-receptors played a part in the insulin-releasing system. On the other hand, the actions of isoproterenol were very complicated. Isoproterenol accompanied with phentolamine provoked a sudden and short rise in the IRI level and produced a sustained fall in the IRI level thereafter. The increasing curve for the IRI level after glucose application was kept unchanged even during the treatment with isoproterenol. Since the initial sudden rise produced by isoproterenol resembled the 1st phase response, and this 1st phase response was thought to depend on the D-cell function, the sympathetic $\beta_2$-receptors were presumed to be distributed on the D-cell membrane. The results described here allow us to analyze the results from the dopamine experiment. Histochemical studies indicate that the endocrine cells of the pancreas islets contain dopamine and that dopamine affects both in vitro glucose-induced insulin secretion and in vivo basal insulin release. Drugs that are analogues of dopamine alter serum insulin concentrations. Dopamine receptors were distributed very characteristically on the B-cells and D-cells. After the treatment with dopamine, the 1st phase response totally disappeared and the 2nd phase response was partially suppressed. The effect of dopamine on the 1st phase response was blocked by propranolol or domperidone (DA$_3$-antagonist). The action of somatostatin was found to be nearly the same as that of dopamine. The somatostatin's action was blocked by phentolamine, but not by propranolol. Since
somatostatin is known to play a physiological role as an information-messenger from the D-cells to the B-cells, it was naturally presumed that phentolamine interfered with the somatostatin action at a point nearer to the B-cell and that propranolol interfered with the dopamine-action at a point proximal to the D-cell, as illustrated in Fig. 7. Domperidone also blocked the dopamine-action. Consequently, these results revealed that the dopamine receptor was prejunctional and was located on the varicosity of the sympathetic β2 neuron, which innervates the D-cells (Table 1). Apomorphine (D1-receptor) did not suppress the 1st phase response and suppressed the 2nd phase response only partially. Thus, it is possible that a small amount of D1-receptors are distributed on the B-cell membrane. As the summary, presumable distribution of catecholaminergic receptors in the islets is shown in Fig. 8.

Itôh et al. and Arneric et al. have previously suggested that since phentolamine blocked the inhibitory action of dopamine, and yohimbine blocked some synthetic DA agonists, sympathetic α2-receptors would be present in the pancreatic islets. When the D-cells and B-cells were set as a series circuit, α2-blockade would act on the B-cells and dopamine would act on the D-cells. The yohimbine-blockade of the synthetic DA-agonist reported by them may indicate that the synthetic compounds used had many side actions, including the α2-agonistic action.

It is interesting that almost all of the catecholaminergic receptors act to depress the insulin-release from the islet. Among them, isoproterenol acts biphasically, initially it lowers the threshold level in glucose chemoreceptor on the D-cell membrane and later it elevates the threshold level. The present data does not include information about the isoproterenol receptors on the B-cell membrane.

References


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