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Effects of galanin on electrical activity of pancreatic islet cells

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ABSTRACT Intracellular microelectrode technique was used to investigate the effect of galanin on the electrical activity of β cells of Langerhans islets of mice. Galanin 0.15 and 0.3 μmol / L in the perfusion medium inhibited the basic electrical activity induced by glucose 5.5, 11.1 and 20 mmol / L in decreasing the frequency and amplitude of the spikes. In some cells, during galanin inhibition there was a hyperpolarization after the spike. Verapamil (30, 60 and 90 μmol / L), a blocker of voltage– dependent Ca channels, blocked dose–dependently the electrical activity induced by glucose and attenuated the depolarization induced by KCl 50 mmol / L. Galanin 0.3 μmol / L also attenuated the depolarization induced by KCl 50 mmol / L, similar to the effect of verapamil. The results suggest that the effect of galanin on inhibition of the electrical activity of β cell might be due to blocking of voltage–dependent Ca^{2+} channels.

KEY WORDS microelectrodes; galanin; verapamil; islands of Langerhans; peptides

Our previous study reported that galanin inhibited insulin secretion from cultured rat islets[1]. Peptide hormones or neurotransmitters are capable of modulating the activity of a variety of ionic channels. For example, β–adrenergic agonists activate Ca^{2+} channels in myocardial cells [2], somatostatin inhibits Ca^{2+} current in pituitary cell lines[3], and GABA induces inhibition of voltage–dependent Ca^{2+} channels in chicken dorsal root ganglion cells [4]. Insulin secretion is clearly related to the electrical activity generated by the insulin–secreting cell and voltage–dependent Ca^{2+} channels which play a key role in this electrical activity.

This study was to determine the effect of galanin on the electrical activity of mouse β cells, and to study which type of channel regulated by this peptide known to alter insulin secretion.

MATERIALS AND METHODS

Preparation Mice, ♀, ♂, weighing 22.1 ± SD 2.8 g were anesthetized with ip urethane 1 g / kg,. A piece of pancreas was excised and fixed to a 1 cm^2 black rubber in the perfusion chamber. The pancreatic membrane and some acinar tissues covering the islets were then removed, so as to let the islets exposed. The preparation , usually took about 15 min and was perfused with glucose–containing Krebs solution at 37 ℃. The volume of the chamber was 1.8 ml, and the perfusion flow rate was 4–5 ml / min.

Solutions Krebs solution was conti–

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nuously gassed with 95% O_2 and 5% CO_2, pH 7.4. When the concentration of KCl was altered, the concentration of NaCl was adjused to maintain the isosmolarity. Galanin was obtained from Sigma. Verapamil was purchased from Shanghai 10th Pharmaceutical Factory. All values were expressed as the $\bar{x}$ ±SD. Data were compared using t test.

Measurement Microelectrodes filled with potassium citrate 2 mol / L and having a tip resistance of about 100 MΩ were used for recording cellular transmembrane potentials. The membrane potentials were measured between 2 Ag / AgCl electrodes, one was intracellular microelectrode and the other in the perfusion medium. Microelectrode was connected with an amplifier (MEZ-7101). The output of the amplifier was recorded by an oscilloscope (SR 46) with camera. Pancreatic β cell was identified by the characteristic eletric burst pattern induced by perfusion of glucose 11.1 mmol / L [5,6]. The results reported in this paper were obtained from cells, in which the microelectrode could be maintained during at least one change of the external fluid, membrane potential could be measured for more than 1 h, in a favourable condition.

RESULTS

Effect in the presence of glucose When the medium contained glucose 5.5 mmol / L or higher, the intracellular recording of β cells showed membrane potentials of −40.1 ± 1.3 mV (n= 84). Increasing of glucose concentration in the medium caused rapid depolarization, appearance of spontaneous electrical activity with gradual increase in number of spikes per burst and termination with continuous spikes.

In the experiment illustrated in Fig 1, galanin 0.3 μmol / L was added to the perfusion solution when bursts were induced by glucose 11.1 mmol / L. After galanin perfusion the bursts activity disappeared and the spikes frequency decreased gradually. Fig 1 E showed an expanded time scale of the Fig 1 B in which galanin attenuated the amplitude of the spikes and induced hyperpolarization after the ending of the spike.

The inhibitory effect of galanin 0.3 μmol / L on the electric activity of β cells varied in response to graded concentrations of glucose in the perfusion medium was illustrated in Tab 1. While the frequency of spikes increased with glucose concentration raised from 5.5 to 27.7 mmol / L, significant inhibitory effect of galanin on the frequency of spikes was noted.

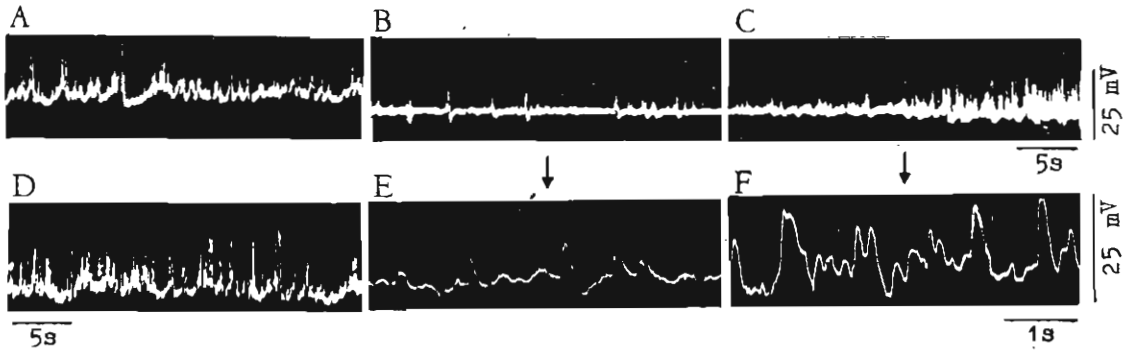

Fig 1. Effects of galanin 0.3 μmol / L on the electrical activity in the presence of glucose 11.1 mmol / L. A) Normal burst activity induced by glucose 11.1 mmol / L. B) 14 min after galanin 0.3 μmol / L. C) & D) 10 & 20 min after removal of galanin. E) Fast record of B). F) fast record of C). All records were from the same cell.

Tab 1 Spikes frequency changes induced by galanin 0.3

Tab 1 Spikes frequency changes induced by galanin 0.3 μmol / L in the presence of glucose. ***$P < 0.01$.

Glucose (mmol / L)	Cells	Spikes / s Control	Galanin
5.5	3	6.0 ± 0.7	0.46 ± 0.2 ***
11.1	5	7.1 ± 4.9	5.4 ± 3.3 ***
27.7	4	9.5 ± 1.1	2.8 ± 0.8 ***

Effect in the absence of glucose In the absence of glucose, the membrane potential were more negative than that in the presence of glucose, reaching a value of −59 ± 5 mV (n = 4), and no spike activity was seen (Fig 2 B). Galanin had no effect on the membrane potentials of β cell in the glucose-free medium (Fig 2 C). When glucose was restored to the medium with galanin, the spikes reappeared (Fig 2 D) with a frequency lower than that of the control (Fig 2 A).

Mechanism of the inhibitory effect of galanin Fig 3 showed the effect of verapamil 30 − 90 μmol / L on the electrical activity induced by glucose 11.1 mmol / L. The plateau usually superimposed with spikes, but the burst activity was progressively blocked and the spike frequency was decreased dose-dependently.

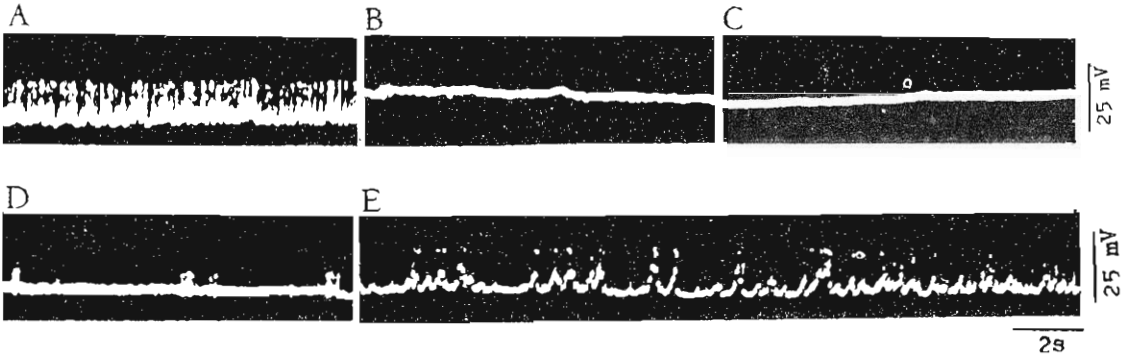

Fig 2. Effects of galanin 0.15 μmol / L on β cell membrane potentials with or without glucose 5.5 mmol / L in the medium. A) Normal burst activity induced by glucose . B) 10 min after perfusion of glucose-free solution. C) 11 min after perfusion of glucose-free solution containing galanin . D) 10 min after perfusion of glucose containing galanin. E) Burst activity induced by glucose after removing galanin. All records were from the same cell.

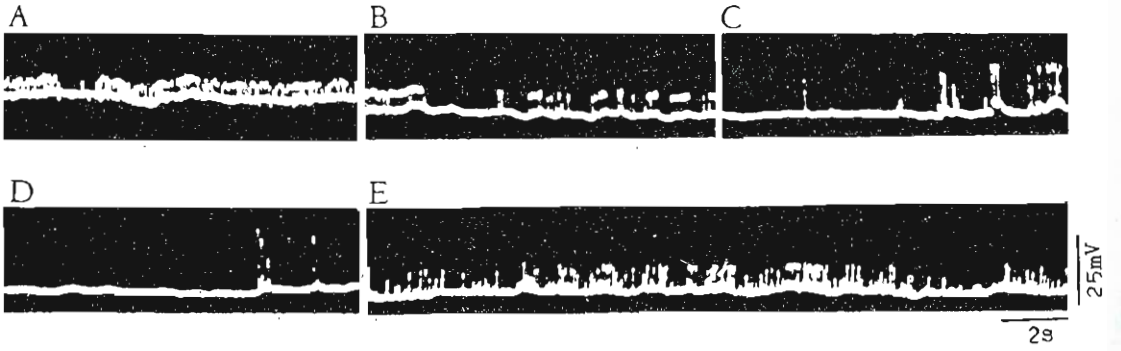

Fig 3. Effect of verapamil on the glucose 11.1 mmol / L induced electrical activity. A) Burst activity induced by glucose. B) 14 min after perfusion of verapamil 30 μmol / L. C) 14 min after perfusion of verapamil 60 μmol / L. D) 14 min after perfusion of verapmil 90 μmol / L . E) After removal of verapamil, burst activity induced by glucose again. All records were from the same cell.

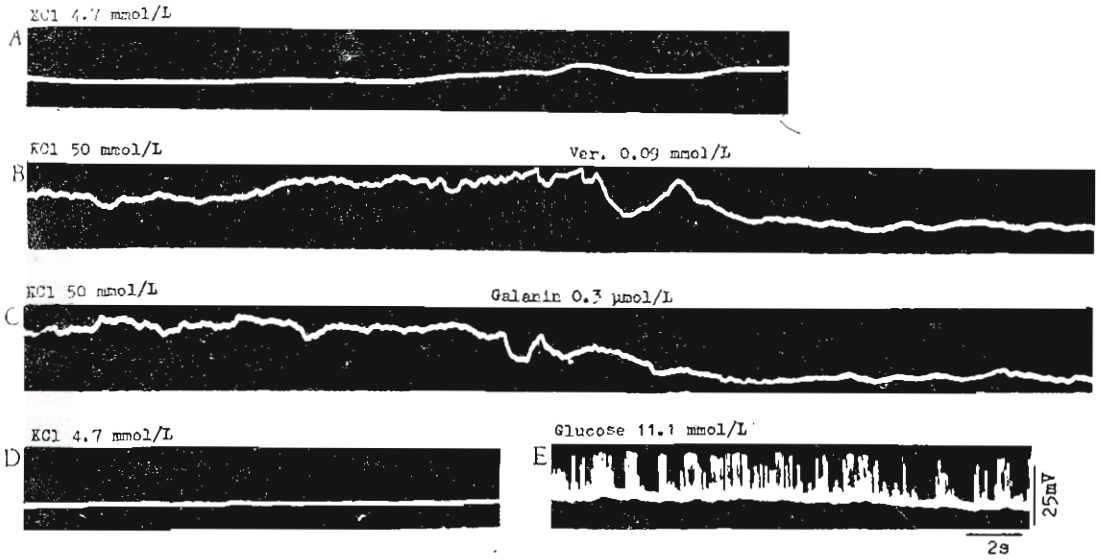

Fig 4. Effects of verapamil and galanin on the membrane potential of β–cell in the KCl 50 mmol / L medium. A) Membrane potential induced by KCl 4.7 mmol / L and glucose 2.8 mmol / L medium. B) Verapamil 90 mmol / L inhibited the depolarization induced by KCl 50 mmol / L. C) Galanin 0.3 μmol / L inhibited depolarization induced by KCl 50 mmol / L. D) 10 min after perfusion of KCl 4.7 mmol / L without verapamil and galanin in the presence of glucose 2.8 mmol / L. E) 11 min after perfusion of glucose 11.1 mmol / L and KCl 4.7 mmol / L. All records were from the same cell.

In the experiment illustrated in Fig 4, as the extracellular KCl concentration was up to 50 mmol / L, a marked depolarization of membrane potentials was observed . Both verapamil 90 μmol / L and galanin 0.3 μmol / L, attenuated similarly the depolarization induced by KCl 50 mmol / L.

DISCUSSION

Verapamil produced a marked reduction in the transmembrane Ca^{2+}conductivity of cardiac muscle but without major change in permeability to other ionic species [7]. It seemed therefore that verapamil might be a useful analytical tool for assessing the importance of Ca^{2+} flux in the electrical activity induced by glucose in islet cells. A number of previous studies using isolated islets suggested that glucose activates insulin secretion by increasing Ca^{2+} flux via an effect on voltage–dependent Ca^{2+} channels [8,9]. In the present study, we found that verapamil dose–dependently inhibited the spike frequency produced by glucose. Devis's work established that verapamil dose–dependently inhibited the first–phase insulin secretion [10]. It seems that verapamil–sensitive Ca^{2+} channels were important in regulating the sustained release of insulin [11].

Our study and Matthews' work[12] showed that perfusing high K^+ medium depolarized islet cell membrane, but there was no basic spike in most conditions. A transient electrical activity might be observed in a few cells during the initial rapid depolarization. The spike discharge usually did not occur because the trigger mechanism was presumably inactivated by the rapid rate of membrance depolarization to a point beyond the critical threshold level [12]. Boyd reported that glucose or depolarization of the B cell with K^+ 40 mmol / L stimulated a monophasic release of insulin directly proportional to the extra cellular Ca^{2+} concentration [9].

Our finding revealed that galanin induced hyperpolarization after the ending of the single spike. This hyperpolarization might result from the activation of galanin-sensitive K^+ channels. Galanin also attenuated the depolarization induced by KCl 50 mmol / L, and showed the same effect with verapamil. The galanin-induced decrease in electrical activity might be due to a decrease in Ca^{2+} flux through Ca^{2+} channels and, as a result of decrease in insulin secretion. It also explained why the galanin was hyperglycemic[13]. Our previous work showed that the inhibitory effect of galanin on insulin secretion was determined by the presence of glucose in cultured rat islet. This was consistent with the present experiments performed on mice islets observing the electrical activity of β cell, which indicated that galanin had no effect on the membrane potentials in glucose-free perfusion medium.

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甘丙肽对胰岛细胞电活动的影响

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提要 采用记录细胞内电位的方法, 观察甘丙肽(galanin)对胰岛 β-细胞电活动的影响. 灌流液中分别加入甘丙肽 0.3 和 0.15μmol / L 均可明显抑制由葡萄糖引起的兴奋性 β-细胞电活动, 表现为锋电位频率减少及锋电位幅度减少; 有些细胞在锋电位复极化后可出现超极化现象. 不同浓度的钙阻断剂维拉帕米(verapamil, 30, 60, 90 μmol / L)可抑制 β-细胞的锋电位, 并呈量-效关系. 甘丙肽和维拉帕米均可抑制同一细胞由高 K^+(KCl 50 mmol / L)灌流液引起的膜电位去极化, 并且这两种物质对膜电位去极化的抑制作用存在相同的形式, 结果提示甘丙肽对 β-细胞电活动的阻抑作用可能是通过抑制 Ca^{2+}电压依赖性通道而实现的.

关键词 微电极; 甘丙肽; 维拉帕米; 胰岛; 多肽