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Effects of substance P on electric activity of mouse pancreatic islet cells *in vitro*

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ABSTRACT The effects of substance P (SP), [*D*-Pro^2, *D*-Trp^{7,9}]SP and verapamil on the electric activity of B cells of Langerhans islets of mice were investigated. Addition of SP 100 nmol · L^{-1} to the perfusion medium stimulated spontaneous electric activity induced by glucose 5.5 mmol · L^{-1} with an increase in frequency and amplitude of the spikes. [*D*-Pro^2, *D*-Trp^{7,9}]SP 50 nmol · L^{-1} reversed the stimulative effect of SP. There was no evidence for an enhancing interaction between SP and acetylcholine 1 μmol · L^{-1}. Verapamil 60 μmol · L^{-1} blocked the stimulative effect of SP. These results suggest that the stimulative effect of SP on the electric activity of B cells may be due to the increase in Ca^{2+} influx through the voltage-dependent Ca^{2+} channels.

KEY WORDS membrane potentials; islands of Langerhans; substance P; [*D*-Pro^2, *D*-Trp^{7,9}]substance P; acetylcholine; verapamil

Polypeptide hormones originating from the gut and pancreas have long been known to be of importance for the regulation of mammalian fuel digestion and metabolism. Among such peptides are somatostatin, SP, vasoactive intestinal peptide (VIP), gastric inhibitory peptide (GIP), neurotensin, and pancreatic polypeptide (PP). Somatostatin, GIP, neurotensin, and PP seem to occur exclusively in endocrine cells of the mammalian gut and pancreas, whereas SP occurs in nerves as well and VIP in nerves only[1-3]. The elucidation of the functional role of most of these polypeptides is still largely in its initial stage and complicated by species difference in the distribution of their cellular origin[4]. In our previous study, it was established that SP stimulated the insulin secretion in a dose-dependent manner from cultured rat islets[5]. The aim of the present investigation was to assess the effect of SP on the electric activity of mouse B cell. An antagonist, [*D*-Pro^2, *D*-Trp^{7,9}]SP, and a blocker of voltage-dependent Ca^{2+} channels, verapamil, were used in an attempt to elucidate the possible mechanism of SP acting on the pancreatic B cell.

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MATERIALS AND METHODS

The technique *in vitro* has been described[5]. Mice weighing 23 ± SD 2.9 g were anesthetized with urethane 1 g · kg^{-1} ip. Partially dissected mouse islets of Langerhans were continuously perfused at 37℃ with a modified Krebs' solution (NaCl 110, $NaHCO_3$ 25, KCl 5, $CaCl_2$ 2.56, and $MgCl_2$ 1.13 mmol · L^{-1}). The pH was maintained at 7.4 by continuously bubbling (95% O_2 + 5% CO_2). SP and [*D*-Pro^2, *D*-Trp^{7,9}]SP were obtained from Sigma. Acetylcoline (ACh) and verapamil was purchased from the Shanghai 3rd Reagent Factory and the Shanghai 10th Pharmaceutical Factory respectively.

The membrane potentials were measured between two Ag-AgCl electrodes, one in contact with external medium and the other contacting a potassium citrate 2 mol · L^{-1} solution within glass microelectrode. Microelectrodes were connected with an amplifier (MEZ-7101). The output of the amplifier was monitored on an oscilloscope. All figures were obtained from the output of polygraph system WI-641 (Nihon Kohden). All values were expressed as $\bar{x}$ ± SD. Data were compared using *t* test.

RESULTS

Effect of SP on electric activity of B cells with / without glucose The resting membrane potentials of B cells showed −43 ± 6 mV (n = 20). Typical electric activities were indicated in Fig 1A with glucose 5.5 mmol · L^{-1}. After the addition of SP 100 nmol · L^{-1}, the initial effect was an increase in electric activity Fig 1A. The spike frequency increased from 1.8 ± 0.7 spikes · s^{-1} in control to 5.0 ± 2.0 ($n = 11$, $P < 0.01$). The results presented in Fig 1C indicated SP was added to the medium in the absence of glucose. The spike frequency and amplitude were progressively decreased and finally disappeared. It showed the membrane potentials of B cells were depressed in the absence of glucose and SP did not have the excitatory effect.

Effect of [*D*−Pro^2, *D*−Trp^{7,9}]SP on electric activity of B cells The spike frequency of B cell was increased after the addition of SP 100 nmol · L^{-1} (Fig 1A). The stimulative effect of SP was reversed by [*D*−Pro^2, *D*−Trp^{7,9}]SP 50 nmol · L^{-1} (Fig 1B). [*D*−Pro^2, *D*−Trp^{7,9}]SP *per se* did not produce any significant changes on the electric activity of B cell in 5.5 mmol · L^{-1} glucose perfusion medium (Fig 1D).

Effect of ACh and verapamil on electric activity of B cells induced by SP When ACh (1 μmol · L^{-1}) was added to the medium, the spike frequency and amplitude of B cells were increased in the presence of glucose 5.5 mmol · L^{-1}. After a significant increase in spike frequency were induced by SP, ACh was added, the spike frequency were not further increased (Fig 1E, 1F). But Ca^{2+} channel blocker verapamil (60 μmol · L^{-1}) blocked the stimulative effect of SP.

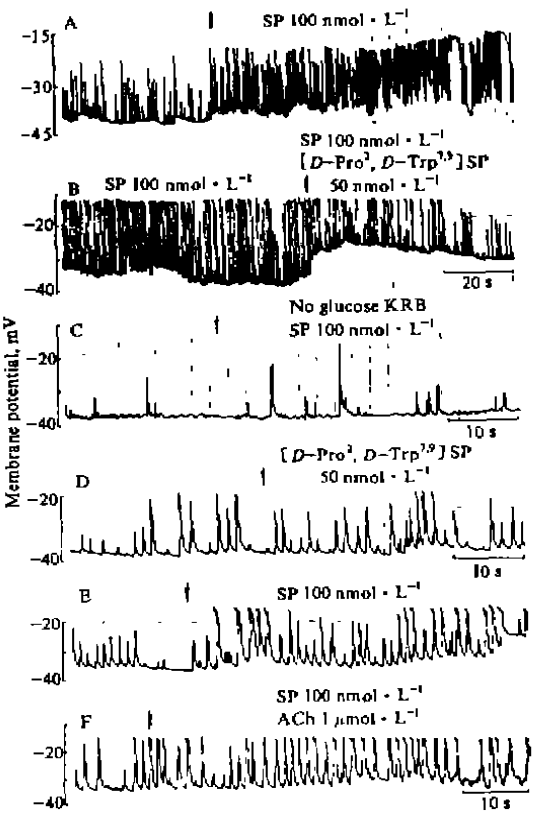

Fig 1. Membrane potentials of B cells as SP, [*D*−Pro^2, *D*−Trp^{7,9}]SP and ACh were added in the medium. All experiments done in the presence of glucose 5.5 mmol · L^{-1}. A) Membrane potentials after SP 100 nmol · L^{-1} B) Membrane potentials after SP 100 nmol · L^{-1} and [*D*−Pro^2, *D*−Trp^{7,9}]SP 50 nmol · L^{-1} in the same cell as A. C) SP 100 nmol · L^{-1} in the absence of glucose. D) [*D*−Pro^2, *D*−Trp^{7,9}]SP only. E) SP 100 nmol · L^{-1} as control. F) SP 100 nmol · L^{-1} together with ACh 1 μmol · L^{-1} in the same cell as E.

DISCUSSION

The results demonstrated that the stimulative effect of SP might act on the voltage-dependent Ca^{2+} channels. The opening of Ca^{2+} channel induces the influx of Ca^{2+}, which triggers insulin release. Ca^{2+} influx through the Ca^{2+}-channel causes a very high concentration of Ca^{2+} in the local vicinity of the channel. Accumulation of Ca^{2+} in the cytosolic compartment, however, leads to the activation of the Ca^{2+}-activited K^+ channel and will repolarize the membrane to block the further opening of the Ca^{2+} channels[6]. In our study, SP could prolong the spike

duration, it seems that SP might lead to accumulate Ca^{2+} in the cytosolic compartment slowly so that membrane potentials would depolarize longer.

According to some authors, the electric activity induced by glucose was enhanced by cholinergic stimulation, and acetylcholine stimulation of insulin release was abolished in Ca^{2+} free medium and blocked by verapamil in Ca^{2+}–containing solution[7,8]. It is therefore reasonable to conclude that the potential of insulin release by cholinergic agents is caused by enhancement of the electric activity of B cells. The stimulation of insulin release by cholinergic agents could be viewed as resulting from a dual mechanism, namely the activation of protein kinase C and the mobilization of intracellular Ca^{2+} [9]. With regard to interaction and coexistence between SP and classical neurotransmitters, so far, no evidence for a coexistence between SP and catecholamines had been obtained[9]. In our study, there was also no evidence for an enhancing interaction between SP and ACh.

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P 物质对小鼠离体胰岛 B 细胞电活动的影响

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提要 用记录细胞内电位方法，观察到 P 物质(SP) 100 nmol·L^{-1} 可兴奋 B 细胞电活动，表现爆发性电位，锋电位和频率均增加，且可被其拮抗剂[*D*-脯^2, *D*-色^{7,9}] P 物质 50 nmol·L^{-1} 所反转，乙酰胆碱 1 μmol·L^{-1} 可兴奋 B 细胞，但与 SP 同时使用并不出现相加效应．维拉帕米 60 μmol·L^{-1} 可阻断 P 物质的效应．提示 SP 兴奋效应可能是通过电压依赖性 Ca^{2+}通道的．

关键词 膜电位；胰岛；P 物质；[*D*-脯^2, *D*-色^{7,9}] P 物质；乙酰胆碱；维拉帕米