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Evidence that muscarinic potentiation of insulin release is initiated by an early transient calcium entry

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The increased insulin release induced by carbamoylcholine (CbCh) in pancreatic islets requires the presence of extracellular Ca^{2+}. Intracellular recordings demonstrate that CbCh produces a transient increase in Ca^{2+} channel activity lasting from 30 to 60 s. Thereafter activity decreased to levels lower than in controls. When extracellular Ca^{2+} was present during this initial period, the stimulatory effects of CbCh were not different from those in which Ca^{2+} was present throughout. These experiments suggest that during muscarinic potentiation of insulin release extracellular calcium is only needed in the first minute.

Ca^{2+} channel; Muscarinic receptor; Insulin secretion; Intracellular messenger; Islet of Langerhans (Pancreatic β-cell)

1. INTRODUCTION

In the pancreatic B-cells, Ca-entry plays a key role in the initiation of the cascade of events that promote insulin secretion. Glucose and other secretagogues such as 2-ketoisocaproate, depolarize the cell by blocking an ATP-sensitive channel [1–3] and this depolarization leads to the opening of voltage-dependent calcium channels. It is generally accepted that Ca^{2+} is one of the intracellular messengers involved in insulin secretion.

The mechanism through which neurotransmitters such as acetylcholine potentiate insulin release is not well-understood. Islet cells possess muscarinic receptors as revealed by binding of [^{3}H]methylscopolamine [4] and [^{3}H]quinuclidinyl-benzilate [5]. It is also accepted that acetylcholine-induced insulin release in isolated pancreatic islets is mediated by an increase in Ca^{2+} influx [6,7]. Estimations of intracellular ionized calcium with fluorescent indicators in an insulin-secreting cell line [8] and ^{45}Ca efflux in islets from normal rats [9] and ob-ob mice [10] suggest that muscarinic receptor activation could generate a calcium transient. However, the relevance of extracellular calcium and calcium channel activity in the full development of the secretory response of cells which display a sustained response to the sustained presence of agonists has not been studied in detail.

The purpose of this paper is to gain insight into how the Ca messenger system operates when the pancreatic B-cell displays a continuous response to the sustained presence of agonist. The specific aims of the present paper are: (a) to monitor calcium channel activity after a challenge with carbamoylcholine and (b) to test whether potentiation of insulin release by carbamoylcholine depends on an early calcium entry.

2. MATERIALS AND METHODS

2.1. *Intracellular electrical activity*

Intracellular electrical activity of mouse islets of Langerhans was measured as described [11]. Net ionic current during the action potential was estimated by assuming that the cell interior is isopotential, thus

$$I_m = -C_m \, dV/dt$$

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where I_m represents the current discharging the membrane capacitor, C_m represents the membrane capacitance and dV/dt the time derivative of the membrane potential. C_m for a single B-cell was taken to be 5 pF [14]. Values of net inward current estimated using this method (5–15 pA) are in the order of those measured with whole-cell clamp [12]. For the analysis of the spike frequency and the variance of the membrane potential fluctuations, records were digitized in data blocks of 1024 points (sampling rate 5 ms/point). Spike frequency is expressed as the inverse of the peak-to-peak interval. The variance of the membrane potential was calculated as described [13]. The modified Krebs solution used had the following composition (mM): 120 NaCl, 25 $NaHCO_3$, 5 KCl, 2.6 $CaCl_2$ and 1 $MgCl_2$ and was equilibrated with a gas mixture containing 95% O_2 and 5% CO_2 at 37°C.

2.2. *Multi-islet perifusion and insulin release*

These were performed as described [11]. The time taken for full exchange of the chamber and of the tubing between the stopcock and the chamber was 20 s. Perifusates were collected and stored at −20°C and later assayed by radioimmunoassay using rat insulin (Novo Research Institute) as standard.

3. RESULTS AND DISCUSSION

3.1. *Carbamoylcholine induces a transient increase in calcium entry*

Fig.1 shows the effect on glucose-induced electrical activity of 100 μM CbCh, a concentration that increases insulin secretion [10]. As described for muscarinic cholinergic agonists, CbCh depolarizes the B-cell and induces firing [14,15]. After exposure to muscarinic agents, pancreatic B-cell develops an initial rapid depolarization with increased rate of firing. Since fast spikes on the plateau are Ca-dependent, Ca-entry should be increased during this period. This is followed by hyperpolarization also accompanied by a decrease in the input resistance (unpublished observation by Sānchez-Andrés and Soria). This suggests the contribution of a potassium permeability. Then there is a slow depolarization with an increase in the in-

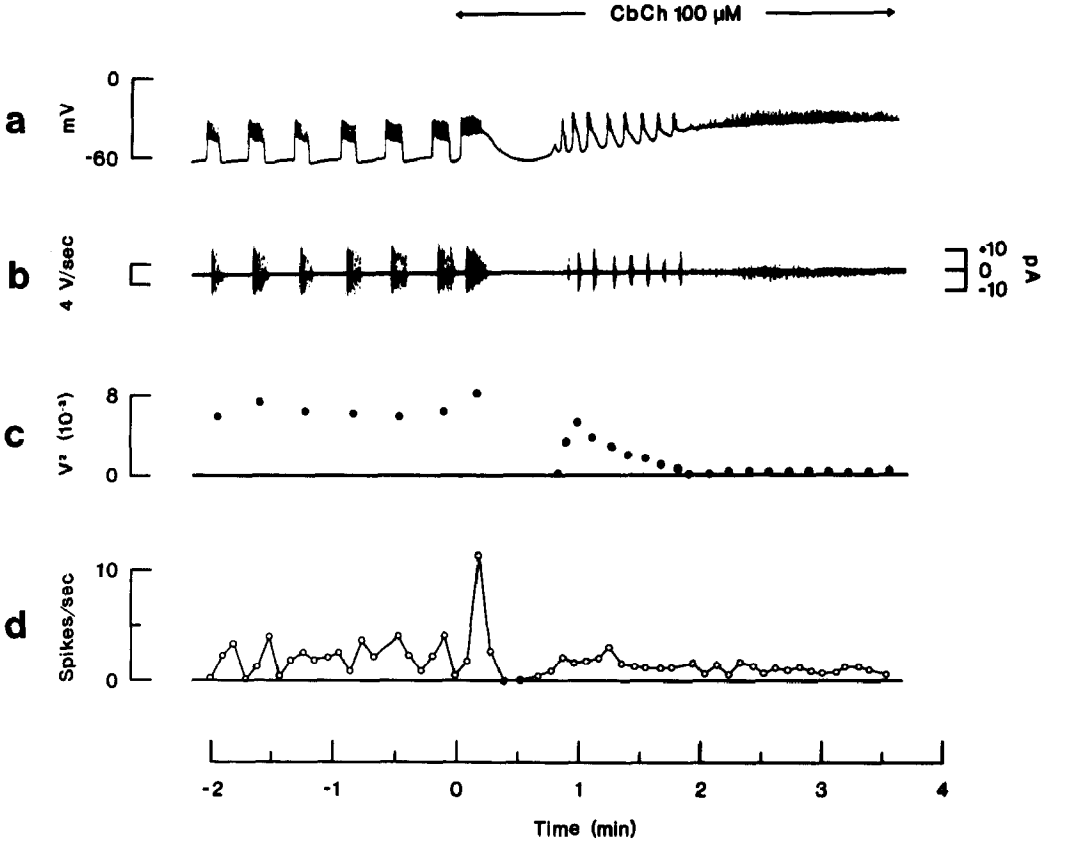

Fig.1. Effect of carbamoylcholine on glucose-induced electrical activity. (a) Effect of 100 μM CbCh; (b) dV/dt; (c) variance; and (d) spike frequency.

put resistance. Burst pattern disappears after 2 min and the cell stays depolarized at the plateau level. This depolarization is, however, different from the one induced by 20 mM glucose in the sense that glucose produces continuous firing whereas during the depolarization induced by muscarinic agents spike frequency decreases after 2–3 min [14].

Ca^{2+} enters the B-cell via voltage-dependent channels; it has also been suggested that Ca-channels may contribute to the slow wave generation and the plateau current. Therefore, we decided to estimate calcium channels activity in three different ways: measuring spike frequency, dV/dt of the spike and the variance of the membrane potential fluctuations which incorporates any change in the membrane potential and has been used as an estimation of calcium channel properties [16]. After exposure to a solution containing CbCh there is a peak in the spike frequency and the variance of the electrical activity which lasts for 30–60 s. The amplitude and the duration of this peak depend on the agonist concentration. After this peak, values of dV/dt, spike frequency and variance decline to the control figures or even below (specially for the higher concentrations). In our experiments high concentration of agonists were selected in order to reproduce the conditions in which other workers have demonstrated the central role of extracellular calcium in the potentiation of insulin release [6,7], the transient efflux of Ca [9,10] and the transient variation of the ionized intracellular calcium measured with Fura-2 [8]. However, this high concentration poses the question of whether non-muscarinic responses may be involved in the effects. Therefore, we have tested the inhibition of the response with muscarinic antagonists. Fig.2 shows that both the early (0–1 min) and late (1–25 min) effects of carbamoylcholine (100 μM) on glucose-induced electrical activity are suppressed by atropine (5 μM).

3.2. *Calcium is only required during the first minute after the challenge with carbamoylcholine (fig.3)*

After a challenge with 1 mM CbCh, insulin release from freshly microdissected mice islets increased to about twice the control values (11.1 mM glucose was present throughout the experiment). This secretion level was maintained while CbCh

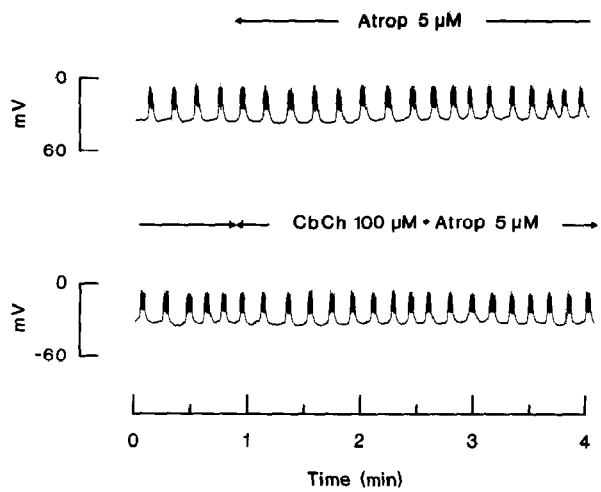

Fig.2. Atropine suppression of CbCh effects on electrical activity.

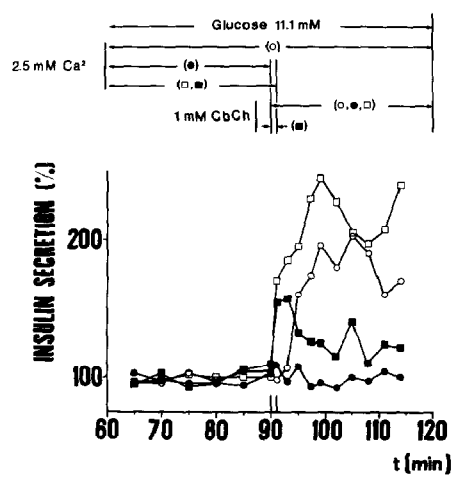

Fig.3. Calcium dependence of muscarinic potentiation of insulin release. Glucose (2 g/l) present throughout the experiment. CbCh (1 mM) was applied after a 90 min period of stabilization in the presence of glucose. (○) Control of the effects of CbCh in the presence of normal calcium. (●) Effect of CbCh in the absence of Ca^{2+}. (□) Ca^{2+} was present only during the first minute of CbCh application. (■) CbCh and Ca^{2+} withdrawn after the first minute. Insulin secretion expressed as a percentage (100%: 0.07 ± 0.03 ng·min^{-1}·islet^{-1}). Secretion in ○ and □ is not different between them and statistically different from ● ($P \leq 0.02$). Secretion in ■ is different from ● during the first 8 min ($P \leq 0.02$). Each point is the mean of 4 experiments. After each experiment islets were challenged with 4 g/l glucose (secretion ranges from 0.14 to 0.34 ng·min^{-1}·islet^{-1}).

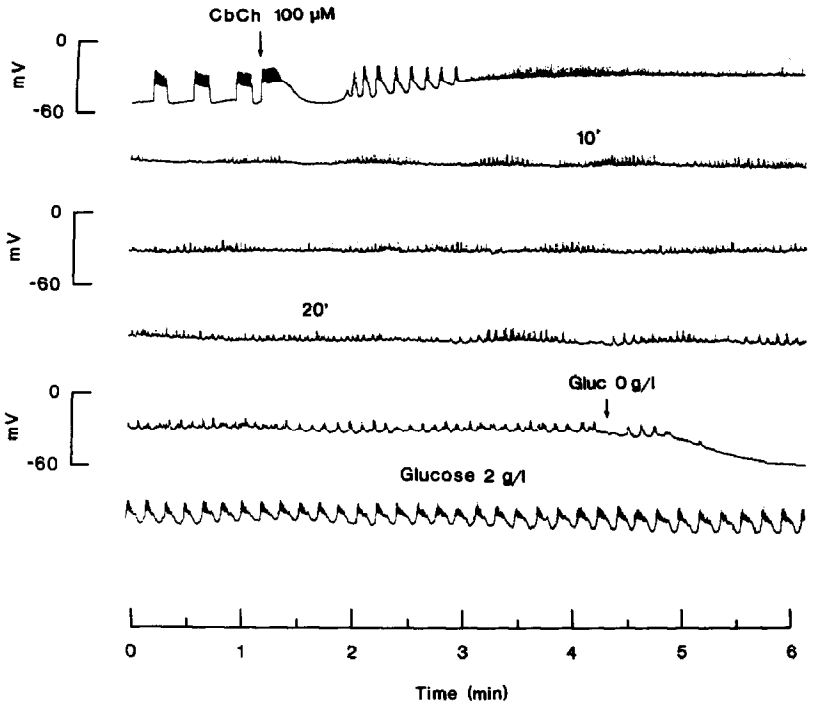

Fig.4. Influence of 100 μM carbamoylcholine on 'steady-state' electrical activity induced by 11.1 mM glucose.

was present. When carbamoylcholine was applied in a calcium free medium (no Ca^{2+}, 1–2 mM EGTA, 3.6 mM Mg^{2+}) no potentiation of insulin release was observed. However, when extracellular calcium (2.5 mM) was present during the first minute of exposure to CbCh, the stimulatory effects were not different from those in which Ca^{2+} was present throughout the experiment. This finding clearly demonstrates that extracellular calcium is only required during the first minute of stimulation with CbCh. When both carbamoylcholine and Ca^{2+} were withdrawn after the first minute, insulin secretion increased for the next 4–5 min and then decayed to Ca-free values (fig.3). Therefore, intracellular mechanisms promoting exocytosis last for 4–5 min after withdrawal of agonists. In experiments in which islets were exposed to CbCh (100 μM) during a prolonged period, the membrane potential followed the same pattern as that observed during the first 3–4 min after the initiation of the effects (fig.4). Spike frequency, dV/dt and the variance of the membrane potential fluctuations stayed at the low figures reported in fig.1 for 30 min.

These experiments demonstrate that extracellular calcium is only required during the first minute of stimulation with muscarinic agonists. This observation, together with the transient responses in ionized intracellular calcium and those in transmembrane Ca^{2+} movements strongly suggest that muscarinic agonist induces a transient Ca^{2+} entry which acts as an early activator of insulin release whereas other messengers might be involved in the full development of the secretory response. Using the terminology coined by Rasmussen and Barrett [17], Ca^{2+} is a mercurial messenger, in that the rise in its concentration in the cell cytosol is transient even in the cells displaying a sustained response. Further experiments are needed to evaluate the role of other intracellular messengers on the neuromodulation of the stimulus–secretion coupling in the pancreatic B-cell.

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