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Muscarinic receptor modulation of glucose-induced electrical activity in mouse pancreatic B-cells

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Acetylcholine (1–10 μM) depolarized the membrane and stimulated glucose-induced bursts of electrical activity in mouse pancreatic B-cells. The acetylcholine effects were mimicked by muscarine while nicotine had no effect on membrane potential. Pirenzepine, an antagonist of the classical M1-type muscarinic receptors, but not gallamine (1–100 μM), an antagonist of the classical M2-type receptors, antagonized the acetylcholine action on glucose-induced electrical activity ($IC_{50} = 0.25$ μM). Bethanechol, an agonist of the classical M2-type muscarinic receptors, was approximately 100 times less effective than acetylcholine in stimulating the electrical activity. In addition, acetylcholine (1 μM) induced a marked increase (25%) in input resistance to the B-cell membrane. The results indicate that acetylcholine exerted its effects on the B-cell membrane by inhibiting K^+ conductance via activation of a muscarinic receptor subtype distinct from the classical M2-type receptor.

Muscarinic receptor; Acetylcholine; Pirenzepine; Gallamine; (Pancreatic B-cell)

1. INTRODUCTION

The autonomic nervous system plays a key role in the regulation of glucose-induced insulin secretion [1,2]. It has been known for some time that both stimulation of parasympathetic nerves and direct application of acetylcholine (ACh) stimulate insulin release [3–5] and glucose-induced electrical activity [6].

Although stimulation of both insulin release and electrical activity by ACh is widely accepted to take place via activation of muscarinic receptors [4,6], the detailed mechanisms by which receptor activation is coupled to hormone release and plasma membrane ion channels are not clear. Stimulated Ca^{2+} entry has been proposed to be an integral part of this sequence of events [6–8]. More recently, however, an important role has also been ascribed to Ca^{2+} mobilization from intracellular stores. This view has gained support from work showing that activation of ACh receptors in the B-cell membrane enhances phosphoinositide turnover, with the production of soluble second messengers (e.g. inositol 1,4,5-trisphosphate) and mobilization of Ca^{2+} from intracellular stores [9–13].

Historically, muscarinic receptors have been differentiated into two subtypes based upon their affinities for the muscarinic antagonist pirenzepine. Thus, classical M1-type muscarinic receptors have high affinity for pirenzepine, while classical M2-type muscarinic receptors have low affinity for this drug [14,15]. Recently, however, analysis of muscarinic receptor genes revealed the existence of four distinct receptor types [16–18].

Activation of classical M1-type receptors has been generally linked to increased phosphoinositide turnover [19]; activation of classical M2-type receptors, on the other hand, has been associated with a decrease in adenylate cyclase activity [19]. While some membrane ionic conductances have also been shown to be under muscarinic receptor control in different systems [20], the particular

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receptor subtypes involved in these interactions are generally unknown.

In the present study, we have characterized the electrical response of the pancreatic B-cell membrane to cholinergic stimulation with ACh, and the subtype of muscarinic receptor involved in this response.

2. MATERIALS AND METHODS

Three- to 5-month-old albino mice were used throughout this study. The animals were given free access to food and water. Pieces of pancreas with partially exposed islets were microdissected and used in an in vitro system as described [21]. The islets were placed in an 80 μl chamber and perifused at a rate of 30 μl/s with a modified Krebs solution containing (mM) 120 NaCl, 25 $NaHCO_3$, 5 KCl, 2.6 $CaCl_2$, 1.1 $MgCl_2$, 11 glucose, which was equilibrated with 95% O_2/5% CO_2 to give a pH of 7.4. Experiments were performed at 37°C.

The islets were impaled with a glass microelectrode in order to record the membrane potential from one B-cell which remained coupled to the other cells in the islet. Potentials were measured with two Ag-AgCl electrodes, one placed in the external solution and the other in the solution filling the microelectrode (1:1 mixture of 1 M K citrate and 3 M KCl). The microelectrodes had tip resistances in the range of 150–300 MΩ. A detailed description of the electrophysiological methods can be found in [22].

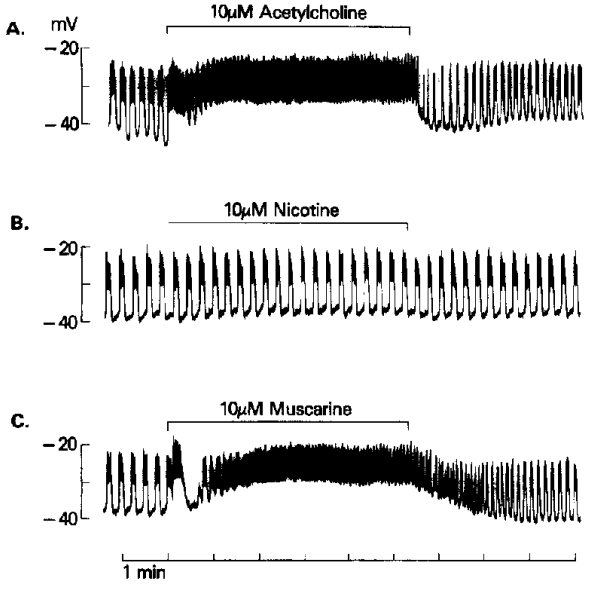

Fig.1. Effects of muscarinic and nicotinic receptor activation on glucose-induced electrical activity. Membrane potential recording from a mouse pancreatic B-cell. The islet was exposed sequentially to ACh (A), nicotine (B) and muscarine (C) as indicated. Glucose (11 mM) was present continuously. Time lapse between each record equal to 5 min.

Cell input resistance was measured by intracellular current injection through the same microelectrode used to measure membrane potential, as described [23].

Acetylcholine chloride, muscarine chloride, nicotine, atropine, gallamine triethiodide, carbamyl-β-methylcholine chloride (bethanechol) (all from Sigma Chemical Company, St Louis, MO) and pirenzepine (kindly provided by Dr J. Daly, NIDDK, NIH) were added to the Krebs solution immediately before application to the islets.

3. RESULTS

3.1. *Acetylcholine stimulation of electrical activity is mimicked by muscarine*

In the presence of glucose (11 mM), the B-cell electrical activity consisted of slow waves (bursts) of membrane potential and rapid fluctuations (spikes) arising from the depolarized (active) phase

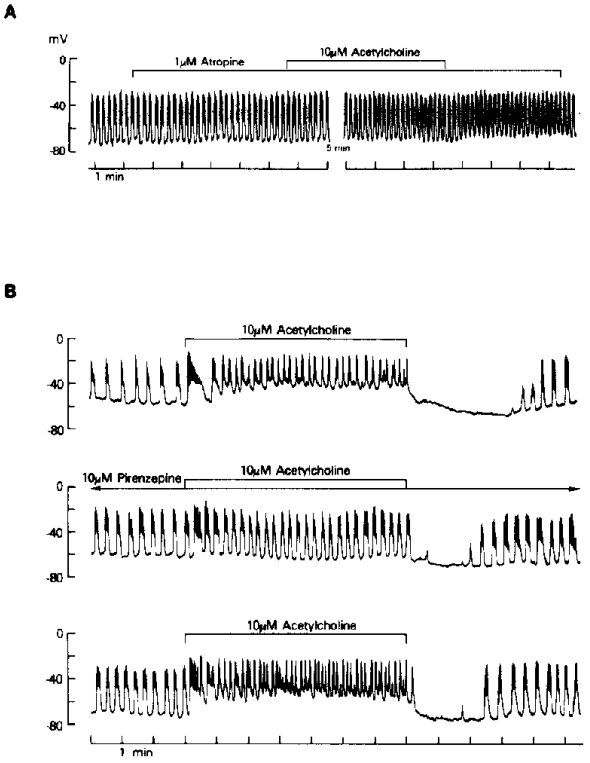

Fig.2. Inhibition of ACh effects on glucose-induced electrical activity by atropine and pirenzepine. (A) ACh was added and removed in the continued presence of atropine as indicated. (B) Islet was exposed to ACh before (upper record), during (middle record) and after (lower record) application of pirenzepine. Preincubation with pirenzepine took 12 min. The third application of ACh occurred 12 min after the removal of pirenzepine. Glucose (11 mM) was present continuously.

of each burst (fig.1A, left). Addition of ACh to the perifusion medium induced a multiphasic change in the pattern of electrical activity consisting of a short period of constant burst activity, which was often (but not always) followed by a period of reduced electrical activity and then by a phase of relatively high burst frequency, about twice that recorded in the control period. During this activity the membrane potential at the silent phase was 10–15 mV more positive compared with that in the absence of ACh. The size of the ACh-induced membrane depolarization was 13 ± 5 mV in 18 different cells. The burst frequency of the steady-state electrical activity remained high as long as ACh was present (longest application time, 20 min). Although not immediately apparent from the records, the average spike frequency was transiently enhanced by ACh (not shown). It may also be seen (fig.1A) that removal of ACh led to membrane hyperpolarization and to a decrease in burst frequency, resulting in the recovery of the pattern of burst activity recorded prior to application of ACh. In most experiments (12 out of 18), this hyperpolarization occurred concomitantly with a transient cessation of electrical activity.

We have carried out experiments aimed at determining whether ACh influenced the electrical activity by activation of muscarinic receptors only, or by activation of both muscarinic and nicotinic receptors. Nicotine (10 μM), a specific agonist of the nicotinic receptor in other preparations, had no effect on glucose-induced electrical activity (fig.1B). On the other hand, stimulation of the muscarinic receptor with a specific ligand, muscarine, mimicked the effect obtained with ACh (fig.1C). We conclude that ACh activates glucose-

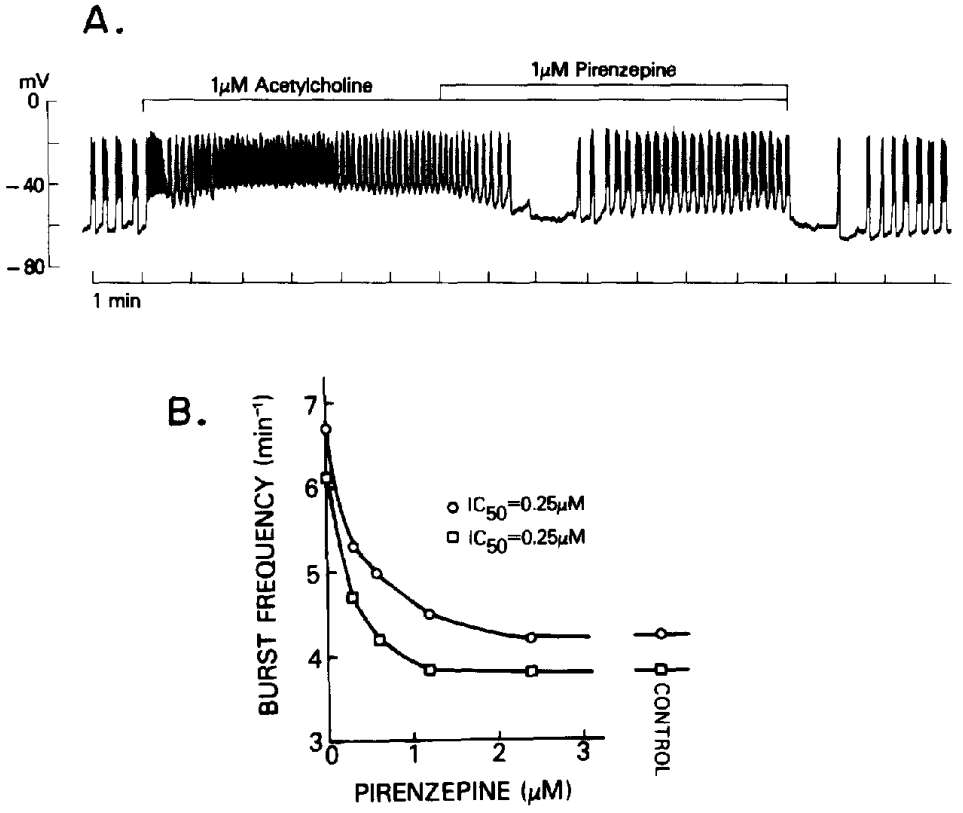

Fig.3. Inhibition of ACh effects on glucose-induced electrical activity by pirenzepine. (A) Pirenzepine was added and removed in the continued presence of ACh as indicated. (B) In two experiments (○, □), pirenzepine was added at increasingly higher concentrations in the continued presence of ACh (1 μM). Time of exposure to each pirenzepine concentration, 6 min. Burst frequency was measured from the last 2 min of continuous recording at each concentration of pirenzepine. Also indicated is the pirenzepine concentration necessary to reduce burst frequency to half of its control value (IC_{50}).

induced electrical activity exclusively via activation of muscarinic receptors.

3.2. *Pharmacological dissection of muscarinic receptor sub-types involved in ACh action*

Atropine (1 μM), a general inhibitor of muscarinic receptors, blocked the stimulatory effect of ACh on the electrical activity (fig.2A). Likewise, preincubation with pirenzepine, a blocker of the classical M1-type muscarinic receptors profoundly inhibited the stimulation of electrical activity brought about by ACh (fig.2B, compare middle and upper records). Pirenzepine not only impaired the ACh-induced depolarization of the membrane, but it also reduced the increase in burst frequency by about 85% (average of two experiments). A third exposure to ACh after pirenzepine removal gave an effect similar to that obtained during the first control application (fig.2B, compare lower to upper record). Thus, the lack of ACh effect in the presence of pirenzepine is not caused by receptor desensitization.

The reversal of the ACh effects by pirenzepine was further investigated in other experiments, by exposing the islets to this antagonist in the presence of ACh. An example of one such experiment is illustrated in fig.3A. Addition of ACh depolarized the B-cell membrane and stimulated glucose-induced electrical activity as described before. Subsequent addition of pirenzepine induced membrane hyperpolarization and a transient cessation of electrical activity, which eventually reappeared with a reduced burst frequency (fig.3A). The dose dependence of the pirenzepine effect was investigated in two additional experiments, by first stimulating the cells with ACh (1 μM), and then adding pirenzepine at successively higher concentrations, up to 2.4 μM (not shown). The steady-state burst frequency was measured at each concentration and plotted against pirenzepine concentration (fig.3B). From these plots, the pirenzepine concentration necessary to reduce burst frequency to control values as about 1.5 μM. Also, the pirenzepine concentration for half-maximal inhibition of ACh-induced stimulation was 0.25 μM.

Gallamine, a potent blocker of heart muscarinic receptors, has an affinity about 12 times higher for classical M2-type muscarinic receptors than for M1-type receptors, and it has accordingly been used as an M2-type receptor antagonist [24–26]. We have attempted to find evidence for the presence of the classical M2-type muscarinic receptors by undertaking a comparative study of the inhibitory properties of gallamine and pirenzepine.

Preincubation of the islets with gallamine (1 μM) for 10 min failed to affect the ACh-induced stimulation of electrical activity (data not shown). In other experiments, addition of gallamine (10 μM) did not affect either the burst pattern or the membrane potential recorded in the presence of ACh (fig.4A). Additional experiments were carried out in which the islets were challenged with increasing gallamine concentrations (1–100 μM), in the presence of ACh (not shown). From these data,

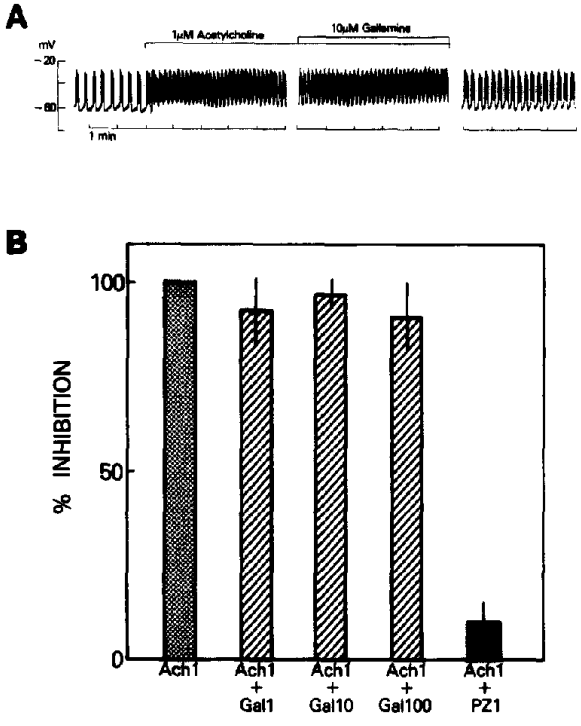

Fig.4. Effect of gallamine on ACh-stimulated electrical activity. (A) Gallamine was added and removed in the continued presence of ACh as indicated. (B) In additional experiments, islets were exposed to gallamine at increasingly higher concentrations (1, 10 and 100 μM) for 5 min in the continued presence of ACh. Bars represent the mean steady-state burst frequency measured from the last 2 min of continuous recording at each gallamine concentration. Results are expressed as % of control values (i.e. in 1 μM ACh). ACh1, 1 μM acetylcholine; Gal1, 1 μM gallamine; Gal10, 10 μM gallamine; Gal100, 100 μM gallamine; PZ1, 1 μM pirenzepine. Vertical lines represent ± SD (n = 3 experiments on different islets).

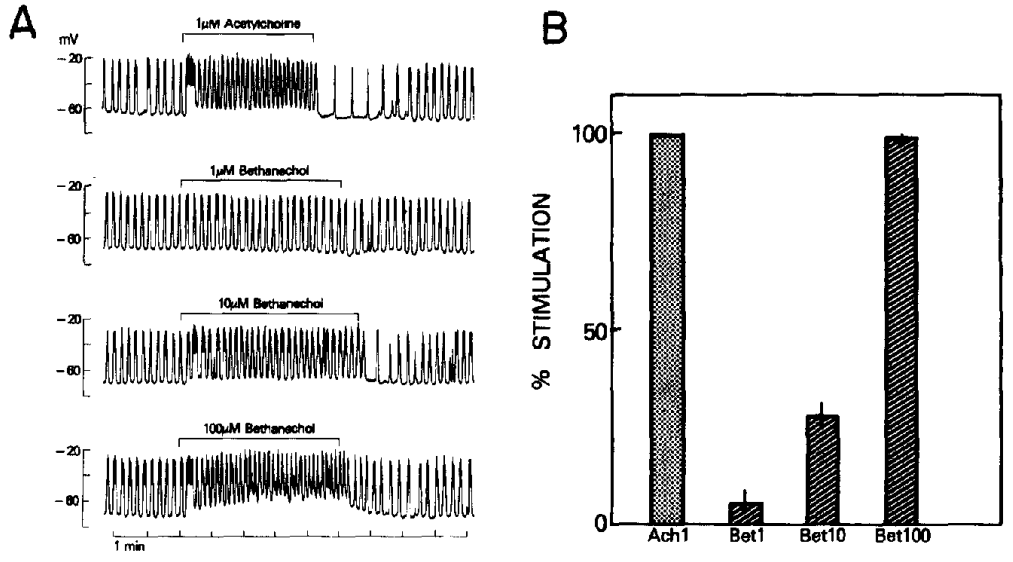

Fig.5. Effects of bethanechol on glucose-induced electrical activity. (A) ACh (upper record) and bethanechol (1, 10 and 100 μM) were added and removed as indicated. Each record is the direct continuation of the previous one. (B) Measurements were made and expressed as for fig.4B. ACh1, 1 μM acetylcholine; Bet1, 1 μM bethanechol; Bet10, 10 μM bethanechol; Bet100, 100 μM bethanechol. Vertical lines represent ± SD (n = 3 experiments on different islets).

the steady-state burst frequency was computed and plotted against gallamine concentration in the bar diagram of fig.4B. For comparison, the inhibitory effect of pirenzepine (1 μM) was also included. While pirenzepine profoundly inhibited the ACh-induced increase in burst frequency, gallamine failed to significantly reduce this parameter, at concentrations up to 100 μM (fig.4B).

We have also compared the relative agonist potencies of ACh and bethanechol, an agonist of the classical M2-type muscarinic receptor [27], on glucose-induced electrical activity. Bethanechol,

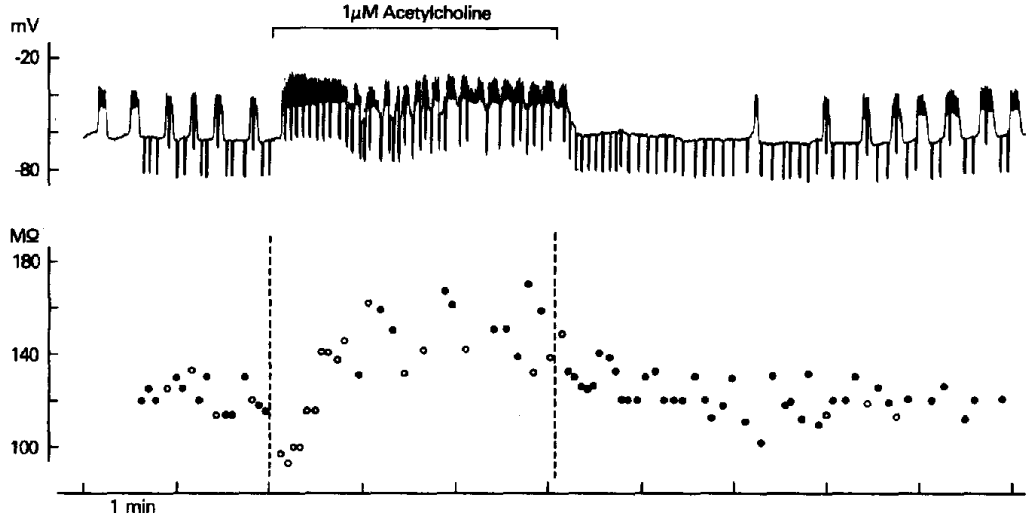

Fig.6. Effects of ACh on cell input resistance. Brief (0.9 s) hyperpolarizing pulses of current (−0.1 nA) were injected into the cell through the same microelectrode used to record membrane potential. Capacitive transients were erased from the record for clarity. Cell input resistance values are plotted as a function of time underneath the record. (•) Current injections along the silent phase of the bursts; (○) current injections along the active phase.

like ACh, induced a dose-dependent decrease in the membrane potential at the silent phase of the bursts as well as an increase in burst frequency (fig.5A). The steady-state burst frequency was computed and plotted against bethanechol concentration in the bar diagram of fig.5B. From these data, it can be seen that bethanechol was 100 times less potent than ACh in stimulating electrical activity.

3.3. *Effect of ACh on B-cell input resistance*

Muscarinic receptor activation may modulate ion channels on the B-cell plasma membrane, thus accounting for the membrane depolarization invoked by ACh. If this were the case, a change in total membrane conductance should become apparent. We have tested this hypothesis by measuring input resistance to the B-cell membrane [23].

As illustrated in the record of fig.6, the membrane responded with downward voltage deflections to injections into the cell of brief pulses of current with a constant amplitude (−0.1 nA) into the cell. The cell input resistance was computed from each voltage deflection and plotted as a function of time under the voltage record shown in fig.6. Exposure to ACh first induced a decrease in input resistance of approximately 20 MΩ, which was rapidly superseded by a slowly developing increase in resistance. This secondary rise, completed in 1–2 min, amounted to about 25% (two experiments).

4. DISCUSSION

The depolarizing and excitatory effect of acetylcholine on the B-cell membrane was mimicked by muscarine but not by nicotine. Furthermore, the membrane response to acetylcholine was blocked by the muscarinic receptor antagonist atropine. We conclude, in agreement with previous reports [4,6], that acetylcholine exerted its effects on the B-cell membrane exclusively via activation of muscarinic receptors.

Our results also show that pirenzepine antagonized ACh action on glucose-induced electrical activity. On the other hand, high concentrations of gallamine, an antagonist of the classical M2-type muscarinic receptor, failed to antagonize the stimulatory effect of acetylcholine. Moreover, bethanechol did not have ACh-like effects in the low concentration range where it supposedly behaves as a selective ligand for classical M2-type muscarinic receptors. Taken together these results indicate that classical M2-type muscarinic receptors are not involved in the action of ACh on glucose-induced electrical activity.

Pirenzepine antagonized the membrane response to ACh with an IC_{50} of 0.25 μM, i.e. a concentration far greater than that required for specific activation of brain M1-type muscarinic receptors [15]. While it is possible that the pirenzepine binding sites on intact islets of Langerhans are less accessible to the drug than those on isolated cells or membranes traditionally used for binding studies, a more plausible explanation could be that a muscarinic receptor type distinct from the classical brain M1- and cardiac M2-subtypes might be involved in the B-cell response to ACh. Recently, analysis of muscarinic receptor genes have indicated the existence of four types of muscarinic receptors. These are the m1, m2, m3 and m4 subtypes according to the nomenclature put forward by Bonner et al. [18]. The m2-subtype, present in heart, has a very low affinity for pirenzepine (IC_{50} = 1.3 μM); on the other hand, the m1- and m4-subtypes have a very high affinity for pirenzepine (IC_{50} = 35 nM) while the m3-subtype shows an intermediate affinity (IC_{50} = 156 nM). It is therefore possible that the B-cell response to ACh might be mediated by the m3-subtype of muscarinic receptor proposed by Bonner et al. [18]. This conclusion is reinforced by the fact that, in different mammalian cell lines, m1- and m3-type muscarinic receptors have been found to be generally coupled to the phosphoinositide messenger system. The pancreatic B-cell activation of the ACh receptor has also been shown to enhance phosphoinositide turnover, with the consequent production of inositol 1,4,5-trisphosphate and mobilization of Ca^{2+} from intracellular stores [9–13].

In many cell systems with functional muscarinic receptors, a slow membrane depolarization is commonly observed to take place in response to receptor activation [29]. While in some cells this depolarization may occur via activation of either a Na^+ permeability or a non-selective cation permeability [30,31], in other cells it may result instead from a decrease in K^+ permeability [29–33]. Several K^+ channels in the plasma membrane of

the pancreatic B-cell have been proposed to play a key role in both the regulation of membrane potential and in the modulation of electrical activity [34]. Thus, it is possible that the excitatory effect of ACh documented in this study may also originate from inhibition of K^+ permeability by muscarinic receptors. Indeed, we found that exposure to ACh was accompanied by a marked increase in input resistance as expected from blockade of K^+ channels.

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