Hormonal activation of single K^+ channels via internal messenger in isolated pancreatic acinar cells

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The mechanism underlying hormonal activation of potassium channels was investigated in pig pancreatic acinar cells by patch-clamp single-channel and whole-cell current recordings. It was shown directly that a peptide hormone belonging to the cholecystokinin-gastrin family, CCK5, can activate single voltage-sensitive potassium channels which can be blocked by tetraethylammonium. The single-channel currents were recorded from electrically isolated cell-attached membrane patches to which the hormone had no access and the activation must therefore involve an intracellular messenger. The hormonal response requires external Ca^{2+} in the isolated membrane-patch area indicating that calcium gating is not directly linked to hormone-receptor interaction.

Pancreatic acinar cell *Single K^+ channel* *Cholecystokinin* *Calcium dependency*

1. INTRODUCTION

Hormones and neurotransmitters open K^+-conductance pathways in a number of different cell types hyperpolarizing the plasma membrane and evoking K^+ release [1]. In mammalian heart acetylcholine-receptor interaction directly triggers opening of K^+ channels [2] whereas hormone or neurotransmitter activation of K^+-conductance pathways in exocrine gland cells operates in a completely different way and is thought to be mediated by the intracellular messenger Ca^{2+} promoting opening of high-conductance channels [1,3–5]. We now report patch-clamp experiments [6] on pancreatic acinar cells in which a cholecystokinin peptide (CCK5) opens single high-conductance and voltage-gated K^+ channels in an electrically isolated patch of plasma membrane to which the agonist has no access. The CCK5-activated single K^+ channels can be blocked by tetraethylammonium (TEA) (5 mM), an inhibitor of the high-conductance, Ca^{2+}- and voltage-activated K^+ channel acting selectively from the membrane outside [7–9] and the sustained messenger-mediated opening of single K^+ channels is shown to be dependent on the presence of external Ca^{2+} in the electrically isolated patch area (patch pipette solution).

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2. MATERIALS AND METHODS

The isolation of pig pancreatic acinar cells was carried out as described [3,5,8]. The experiments were done on small cell clusters (2–4 acinar cells) or on single acinar cells.

The techniques used in patch-clamp single-channel or whole-cell current recordings have been described [6,8]. Two types of physiological saline solutions were used. The extracellular Na^+-rich solution contained (mM): 140 NaCl, 4.7 KCl, 1.2 $CaCl_2$, 1.13 $MgCl_2$, 10 glucose, 10 Hepes (pH 7.2), whereas the intracellular K^+-rich solution contained (mM): 145 KCl, 10 NaCl, 1.13 $MgCl_2$, 10 glucose, 10 Hepes (pH 7.2). In some cases the in-

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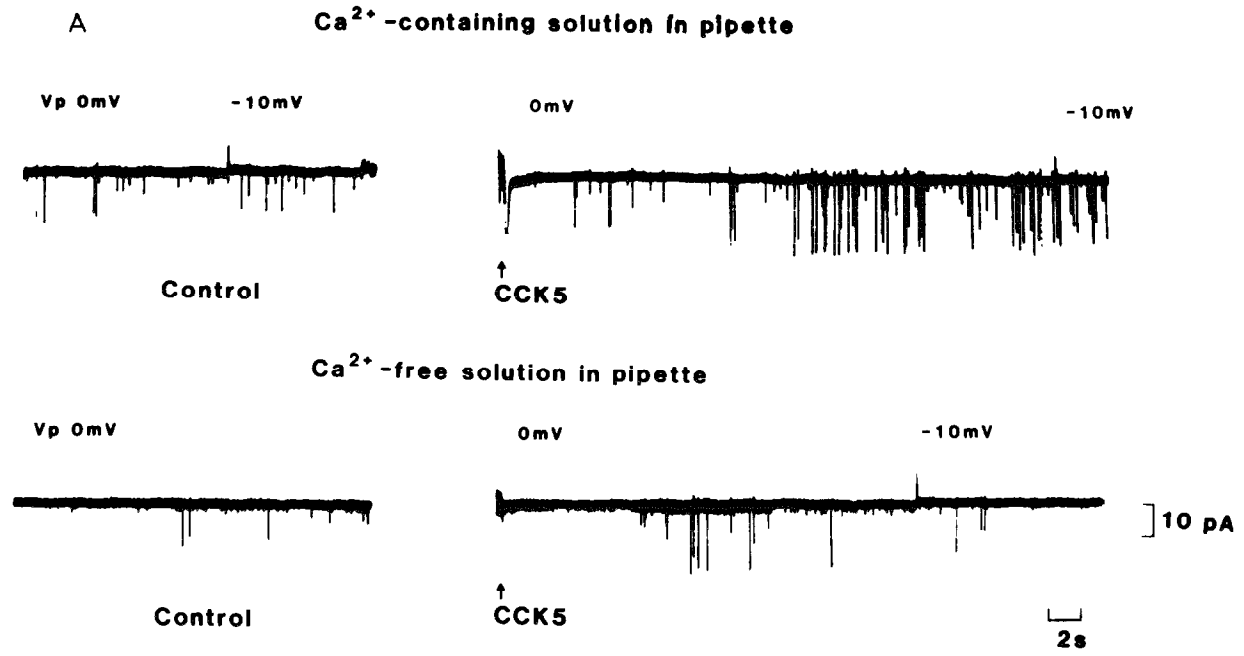


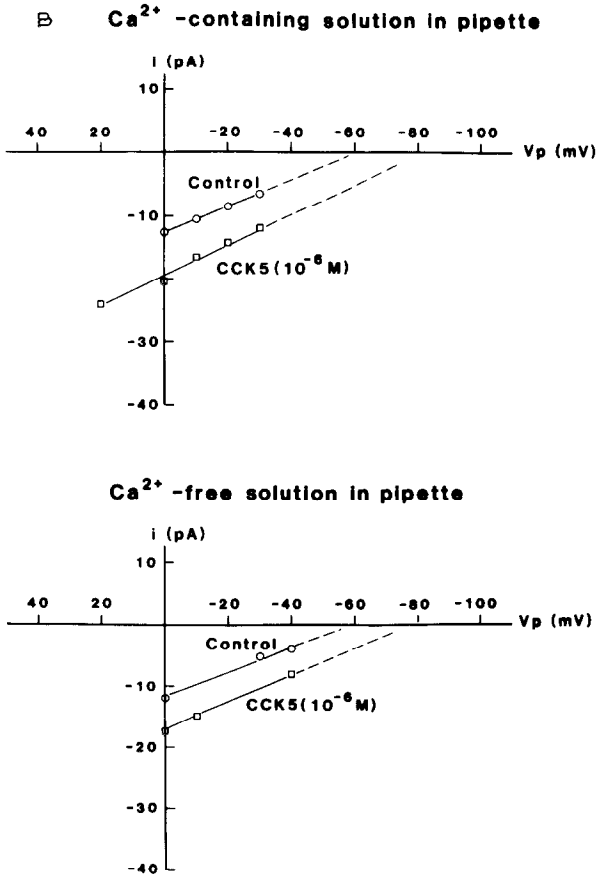
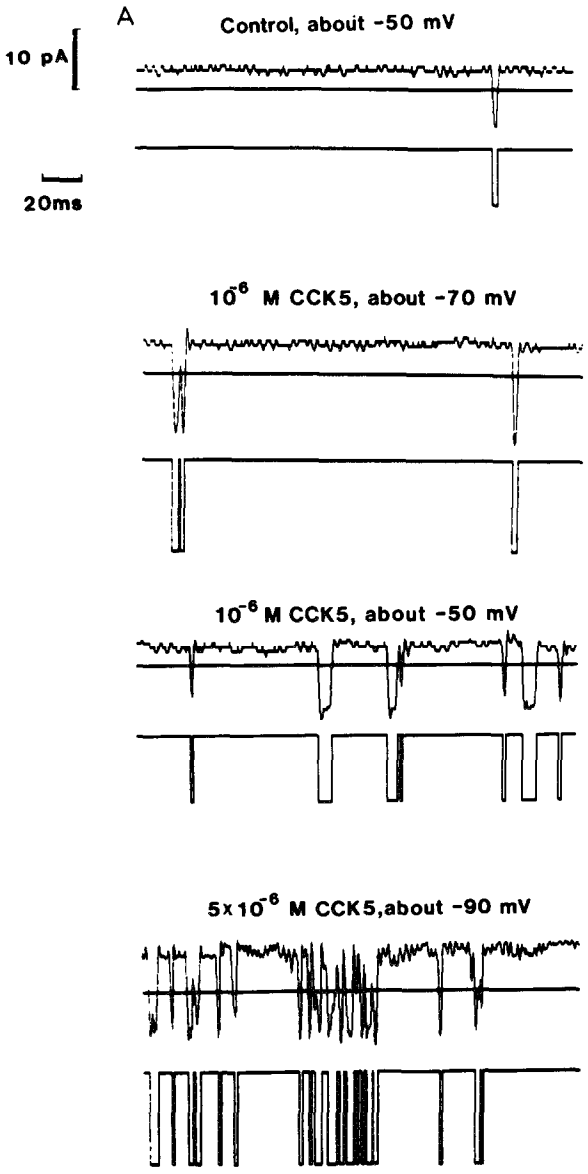


Fig.1. Single-channel current recording from cell-attached pig pancreatic acinar membrane patches. The recording pipette was filled with the intracellular K^+-rich solution with or without Ca^{2+} as indicated. The bath contained the extracellular Na^+-rich solution. (A) Single-channel currents before and after adding CCK5 (pentagastrin) to the bath solution to attain a concentration of 10^{-6} M. In both traces the pipette voltage (V_p) was kept at 0 most of the time (i.e. the single-channel currents were recorded at the spontaneous membrane potential of the cells) but where indicated V_p was changed to -10 mV. (B) Plots of single-channel current amplitude as a function of V_p for the 2 experimental situations.

tracellular solution contained 2.5 mM Ca^{2+} (Ca^{2+}-containing solution); in other cases there was no added Ca^{2+} and 0.5 mM EGTA was present (Ca^{2+}-free solution). The bath was always filled with the extracellular Na^{+}-rich solution and the recording pipette with the intracellular K^{+}-rich solution. Cholecystokinin pentapeptide (pentagastrin, CCK5; ICI) was added to the bath to obtain concentrations of 10^{-6} or 5×10^{-6} M. All experiments were carried out at 22–24°C.



3. RESULTS

Fig.1A shows single-channel currents recorded from cell-attached membrane patches in pig pancreatic acini before and after the addition of CCK5 to the bath. Before the hormone application there were only a few channel opening events seen as brief inward current steps. 10–20 s after adding CCK5 to the bath solution to attain a concentration of 10^{-6} M, there was a marked and sustained increase in the frequency of channel openings if Ca^{2+} (2.5 mM) was present in the pipette filling solution (3 experiments on 3 different cell clusters). In the type of experiment illustrated in the upper part of fig.1A the channel open-state probability (p) before CCK5 application was 0.0018–0.0024

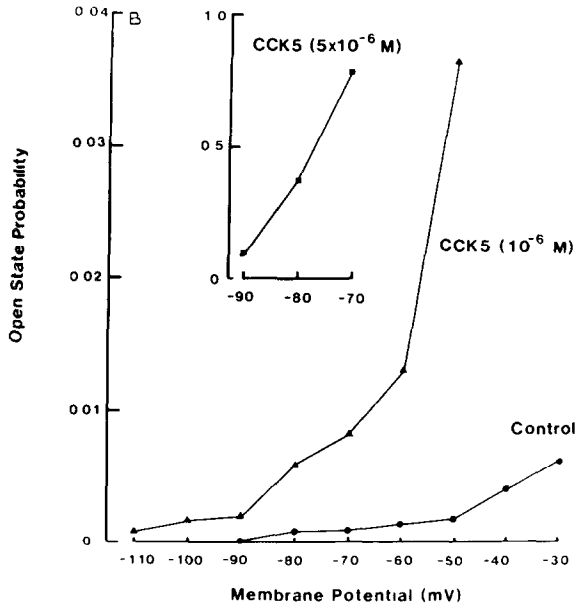


Fig.2. Analysis of channel open-state probability from a single cell-attached membrane patch in an experiment with Ca^{2+}-containing pipette solution. (A) Examples of single-channel current traces together with their idealized current records obtained from computerized threshold analysis of the digitized data. Downwards deflections represent channel openings. (B) The open-state probability as a function of membrane potential in the absence and presence of CCK5 (10^{-6} M). Inset shows the curve obtained from the same experiment in the presence of 5×10^{-6} M CCK5. Note the different ordinate scale. The absolute membrane potential was calculated from the type of plots shown in fig.1B.

whereas in the period after start of stimulation p was 0.01–0.05. In one experiment the CCK5 concentration was raised to 5×10^{-6} M and p thereafter attained a value of 0.79. In contrast, when the recording pipette was filled with an EGTA-containing solution to which no Ca^{2+} had been added, CCK5 had relatively little effect on the open-state probability of the channel (4 experiments on 4 different cell clusters). In the type of experiment illustrated in the lower part of fig.1A p was 0.0020–0.0028 before CCK5 application and in the period after start of stimulation with 10^{-6} M CCK5 p values of 0.0023–0.0039 were obtained. In one experiment the CCK5 concentration was raised to 5×10^{-6} M and p attained a maximal value of 0.0063. The potential in the pipette could easily be changed during these recordings and in fig.1B the amplitude of the single-channel current is shown as a function of the change in membrane potential (pipette voltage). The single-channel conductance calculated from this relationship was 200–250 pS in all the experimental situations. The plots shown in fig.1B can also be used to estimate the absolute membrane potential in the cells under study [3] and by extrapolation one obtains a value of about −60 mV before stimulation and about −80 mV after CCK5 (10^{-6} M) application, both with and without Ca^{2+} in the pipette solution.

Fig.2 illustrates the effect of CCK5 stimulation

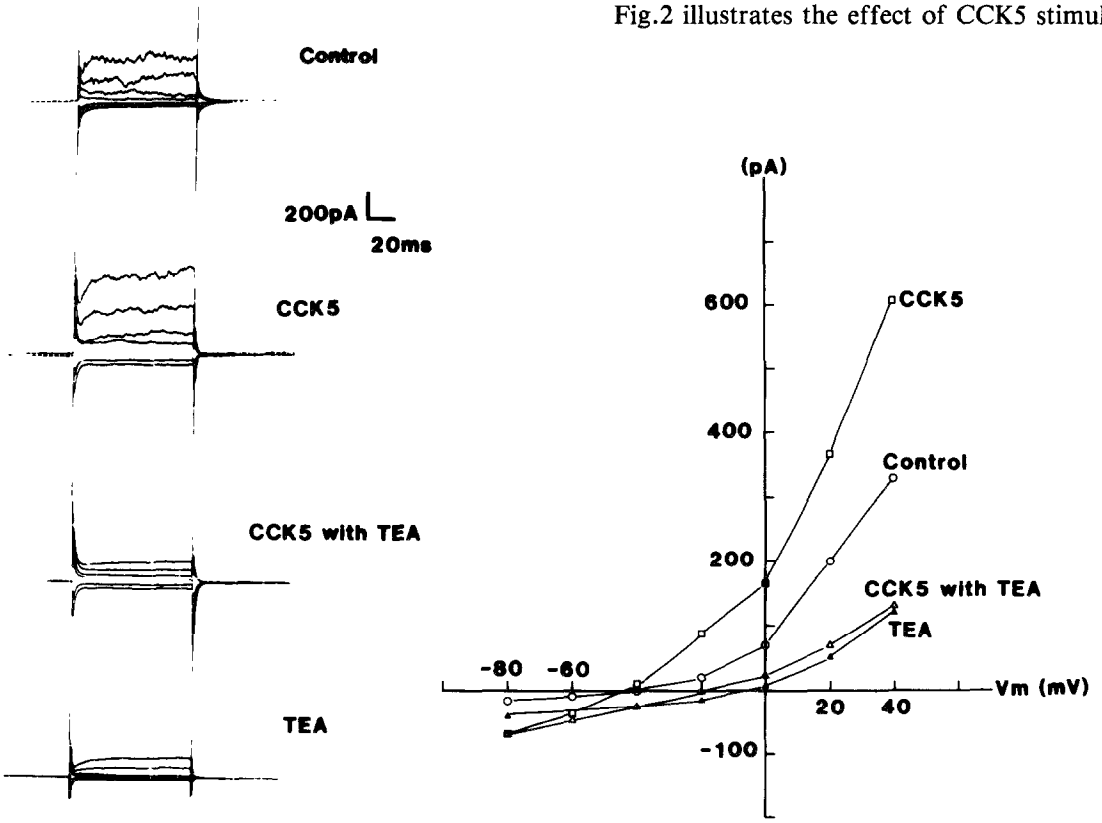


Fig.3. Whole-cell voltage-clamp current recordings from a single acinar cell. The bath contained the extracellular Na^+-rich solution and the pipette was filled with the extracellular K^+-rich solution without Ca^{2+} (containing 0.5 mM EGTA). Currents associated with depolarizing voltage steps are shown as upward deflections (outward current) and with hyperpolarizing steps as downward deflections. The holding potential was −40 mV and 90 ms voltage steps to −20, 0, +20 and +40 as well as −60, −80 and in one case −100 mV were applied. The currents recorded before stimulation (control), 3 min after start of continued exposure to 10^{-6} M CCK5 (CCK5), 3 min after addition of 5 mM TEA still in the presence of CCK5 (CCK5 with TEA) and 3 min after discontinuation of CCK5 stimulation but still in the presence of TEA (TEA) are shown. The relationship between the steady-state currents and the membrane potential in the different experimental situations obtained from the displayed current traces are shown in the graph.

on p in one of the experiments with Ca^{2+}-containing pipette solution. Depolarization increases whereas hyperpolarization decreases p. In the presence of 10^{-6} M CCK5 much higher values of p were obtained than in the control situation at the same or more negative membrane potentials and the voltage sensitivity was very clear. At the higher CCK5 concentration of 5×10^{-6} M a mere 10 mV hyperpolarization from the spontaneous membrane potential of about -70 mV reduced p from about 0.8 to 0.4 (fig.2).

When TEA (5 mM) was present in the solution filling the recording micropipette openings of high-conductance channels were never observed before or after CCK5 application with or without Ca^{2+} in the pipette solution.

After single-channel current recording in the cell-attached conformation using the Ca^{2+}-free pipette solution it is possible to disrupt the isolated patch membrane [6] and establish direct continuity between the pipette and cell interior effectively dialyzing the cell with the pipette filling solution. After a few minutes a new steady state is attained and in this experimental situation the membrane potential was found to be about -40 mV. In the voltage-clamp mode the outward K^+ currents were investigated. Fig.3 shows that the CCK5-evoked increase in the patch-clamp whole-cell K^+ currents was virtually abolished when 5 mM TEA was present in the external bathing solution. Similar results were obtained in 5 experiments on 5 different cells. The resting (unstimulated) K^+ current was also in all cases markedly reduced by TEA.

4. DISCUSSION

We have demonstrated that a peptide secretagogue belonging to the cholecystokinin-gastrin family, CCK5, can activate single large Ca^{2+}- and voltage-activated K^+ channels via an intracellular messenger. The classification of the channel is based on: (i) the high unit conductance (about 200–250 pS) [3,5], (ii) the voltage sensitivity [3] and (iii) the ability of a low external TEA concentration to block openings [8]. Experiments with the whole-cell patch-clamp current-recording technique show that the CCK-elicited increase in the outward K^+ current [5] is blocked by external TEA providing fresh evidence for the hypothesis that this stimulant-evoked voltage-dependent current can be entirely accounted for by the large Ca^{2+}- and voltage-activated K^+ channel. We conclude that the indirect CCK activation of single large Ca^{2+}- and voltage-activated K^+ channels (figs 1 and 2) accounts for the whole-cell K^+ current (fig.3).

The large Ca^{2+}- and voltage-activated K^+ channel has been extensively characterized in excised membrane patches from a variety of cell types [1,3–10]. Channel opening is controlled exclusively by the membrane potential and $[Ca^{2+}]_i$ whereas changes in $[Ca^{2+}]_o$ have no effect. The external Ca^{2+} requirement in the isolated membrane-patch area in the experiments demonstrating indirect CCK5 activation of K^+ channels (fig.1) cannot therefore be explained by the action of Ca^{2+} on the external site of the channel but must be due to an influence on $[Ca^{2+}]_i$. Hormones and transmitters seem to mobilize intracellular Ca^{2+} via the intracellular messenger inositol trisphosphate ($InsP_3$) [11,12] and this substance acts on the endoplasmic reticulum membrane by opening a Ca^{2+} pathway [13,14]. However, many hormone and transmitter-evoked responses in exocrine gland cells are dependent on extracellular Ca^{2+} [15,16] as documented for the stimulant-evoked hyperpolarization and increase in outward K^+ current in pig pancreatic acinar cells [3,5] and pancreatic secretagogues increase unidirectional Ca^{2+} flux into acinar cells [17]. Our experimental procedure may emphasize the external Ca^{2+} dependence by physically moving the isolated membrane patch away from intracellular Ca^{2+} stores and Ca^{2+} released in the pancreatic cell by the messenger following stimulation may not easily penetrate into the patch area due to restrictions of intracellular Ca^{2+} diffusion [18]. $InsP_3$ or another messenger may diffuse more easily than Ca^{2+} and could perhaps mediate the CCK-evoked Ca^{2+}-controlled channel opening by allowing Ca^{2+} influx raising $[Ca^{2+}]_i$ locally.

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