Direct Control of a Large Conductance K⁺-selective Channel by G-Proteins in Adrenal Chromaffin Granule Membranes

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Abstract. We report here the presence of a Ca²⁺-independent K⁺-channel of large conductance in adrenal chromaffin cell secretory vesicle membranes which is controlled by inhibitory as well as stimulatory heterotrimeric GTP-binding proteins. Using antibodies against specific α subunits for immunoblot analysis, we were able to identify the presence of the inhibitory G₁₂ and G₁₃ subtypes, as well as the stimulatory G₂ and G₄ subtypes, but not G₁₁ in adrenal chromaffin granules. Furthermore, functional analysis of the K⁺-channel incorporated into planar lipid bilayers showed that GDPₛ and GTPₐₛ have opposite effects on channel activity inducing interconversions between a low and a high open-probability state. Consistent with these findings, the same antibodies antagonized the effects of the nonhydrolyzable analogues on the open probability of the K⁺-channel.

Key words: K⁺-channel — G-protein — Secretory vesicles — Antibodies — GTPₐₛ — GDPₛ

Introduction

By allowing intact secretory granules to fuse with phospholipid bilayer membranes, we have previously detected the presence of a Ca²⁺-independent K⁺-selective channel of large conductance (150–300 pS) in the vesicle membranes from adrenal chromaffin cells (Arispe, Pollard & Rojas, 1992). Since in the majority of the K⁺-channel incorporations the open probability (Pₒ) was found to be rather low, we concluded that in our chromaffin granule preparation the K⁺-channel was under tonic inhibition by an undetermined mechanism.

G-proteins are responsible for the coupling of cell membrane receptors to membrane resident ion channels or enzymes (Cerione et al., 1986; Yatani et al., 1988; Brown & Birnbaumer, 1990; Spiegel, Shenker & Weinstein, 1992; Brown, 1993), and there is now growing evidence that the distribution of G-protein is not limited to plasma membranes (Carlson et al., 1986; Barr et al., 1991; Donaldson et al., 1991; Carrasco, Sierralta & De Mazancourt, 1994; De Mazancourt, Goldsmith & Weinstein, 1994). In addition, G₂ immunoreactivity has been detected in granule membranes from adrenal chromaffin cells (Toutant et al., 1987). Therefore, in the present work we asked whether the receptor-channel mechanism, present in the plasma membrane, might also be operative in the chromaffin granule membrane.

We found and report here that different antibodies, each one raised against a specific α subunit of a trimeric G-protein, identified the presence of G₁₂, G₁₃ and G₂ but not in G₁₁ in our highly purified preparation of adrenal chromaffin granules (McKenzie et al., 1988; Simonds et al., 1989; Spiegel et al., 1990; Spiegel, 1991; Spiegel, Shenker & Weinstein, 1992). Furthermore, functional analysis of the K⁺-channel incorporated into a planar-lipid bilayer membrane showed that the nonhydrolyzable analogues guanosine 5'-O-(2-thiodiphosphate) (GDPₛ) and guanosine 5'-O-(3-thiotriphosphate) (GTPₐₛ) had opposite effects on the open probability of the channel. Finally, we found that the same antibodies used in the immunoblot analysis could either activate or inhibit the channel in much the same way as GDP and GTP analogues. We conclude that the activity of the chromaffin granule large conductance K⁺-channel is kept under control by direct action of both inhibitory and stimulatory G-proteins on the channel.
Materials and Methods

BIOCHEMICAL PROCEDURES

The methods which were used here have been described elsewhere (Arispe et al., 1992). In brief, chromaffin granules were prepared from bovine adrenal medulla tissue by homogenization in 0.3 M sucrose and purified in a metrizamide step gradient, between densities 1.120 and 1.104 g/cm³ as previously described (Pollard et al., 1979a,b; Brocklehurst & Pollard, 1989). The chromaffin granules were found to be substantially purified from contaminating mitochondria, plasma membranes, and lysosomes.

The C-terminal decapeptides of α₆, αᵣ₁, αᵣ₂, yeast GPI₁₄ (the product of GFA₁), the internal decapeptides of αᵣ₁, αᵣ₂ were synthesized (Doherty et al., 1991), conjugated to keyhole limpet hemocyanin with glutaraldehyde, and injected into rabbits. Some antisera were affinity purified on Affi-Gel 15 columns (Bio-Rad) containing the corresponding immobilized peptide (McKenzie et al., 1988; Simonds et al., 1989a,b; Spiegel et al., 1991; Spiegel et al., 1992). The protein concentration was determined by the Bradford method.

For immunoblot, P₂ membranes and chromaffin granules (150–300 µg protein) were diluted 1:1 in 2× Laemmli buffer and resolved using a SDS-polyacrylamide gel (10% acrylamide, 0.13% bisacrylamide), transferred to PDVF membranes (Millipore) and immunoblotted with 125I-labeled protein A (Amersham).

BILAYER MEMBRANES AND CHANNEL RECORDING

The experimental chamber (made of plexiglass) consisted of two compartments separated by a thin teflon film. Antibodies and reagents were added directly to either the cis or trans side. After each addition, solutions on both sides were simultaneously mixed by two teflon-coated magnetic stirrers placed in a restricted space at the bottom of each compartment. Ag/AgCl pellet electrodes were immersed in a small pool containing 0.5 M KCl and were electrically connected to the solutions in each compartment via agar bridges (2% agar in 0.5 M KCl). Single-channel currents were recorded using a patch clamp amplifier (Axopatch-lD, equipped with a CV-4B bilayer headstage, Axon Instruments) and were stored on magnetic tape using a PCM/VCR digital system (Digital-4, Toshiba) with a frequency response ranging from DC to 25,000 Hz. Records were made from playbacks through a low-pass filter (8-pole Bessel 902 LPF, Frequency devices) set in the range from 200 to 500 Hz (Arispe et al., 1992).

To fuse intact granules with a lipid bilayer we prepared a suspension of the intact vesicles in a KHEpes solution (in mM: 200 KHEpes, pH 7.4). Ion channels present in secretory granules from adrenal chromaffin cells were incorporated into a bilayer by adding a small volume (5–10 µl) of this suspension of intact vesicles to a different KHEpes solution (either 200 or 400 mM). To facilitate membrane fusion, CaCl₂ was added ([Ca²⁺] = 200 µM measured with Ca²⁺-sensitive electrode). Granules were added to the solution in the cis side of the compartment, and incorporation occurred directly from the experimental solutions.

PRESENTATION AND STATISTICAL ANALYSIS

Every experimental paradigm included in this paper was repeated at least three times. The channel activity records shown in all the figures are representative examples of the phenomena studied. Open probability Po values were estimated from digitized data (TL-1, 125 kHz module and DMA interface, Axon Instruments, Foster City, CA) using the pClamp 5.5 software. The data base used to construct the histograms ranged from 10⁹ to 3 x 10⁹ well-defined events for each condition.

Results

The large conductance K⁺-channel present in chromaffin granule membranes exhibits two functional states

The large conductance K⁺-channel incorporated into acidic phospholipid planar lipid bilayers exhibits two distinct patterns of activity. As described previously (Arispe, Pollard & Rojas, 1992), in a large proportion of the experiments (162 out of 277 incorporations), K⁺-channel openings were characterized by low open-probability (hop) values at -10 mV (P < 0.3). Fig. 1A depicts the K⁺-channel activity in its hop modality. Channel openings occurred in brief bursts (upward deflections of the trace) and the single channel conductance γ₂hop was 190 ± 6 pS in symmetrical 200 mM KHEpes. Long lasting inter-burst intervals (closed conformation of the channel) were often observed. The other type of channel activity (detected in 115 out of 277 incorporations), was characterized by high open-probability (hop) values (Po > 0.7) at -10 mV and a single channel conductance γ₁hop of 195 ± 7 pS (symmetrical 200 mM KHEpes). As illustrated in Fig. 1B, the channel in the hop modality remained in the open conformation during long lasting periods with frequent and brief transitions to the closed state (downward deflections of the trace at -10 mV; Po = 0.78). Records with two levels of conductance (Fig. 1C) occasionally showed both the hop pattern (first level; γ₂hop = 202 ± 4 pS) and the hop pattern (second level; γ₁hop = 198 ± 8 pS).

The set of records in Fig. 1 clearly shows that spontaneous transitions between the two patterns of channel activity, and in particular from hop to hop, did not occur. For example, open probability Po values remained almost unchanged at ca. 0.041 along the four consecutive 10-min intervals of channel activity in the hop modality. Similarly, Po values along the hop pattern of channel activity also remained fairly constant for at least 20 min (Fig. 1B,C). Thus, if spontaneous conversions from hop to hop are allowed to occur in our reconstituted system, they were not observed in the time frame of the experiments reported here.

K⁺-channel openings in the hop modality occurred in bursts (Fig. 2A: upward deflections of the trace at -20 and -10 mV; downwards deflections at 10 and 20 mV). Long lasting inter-burst intervals (closed conformation of the channel) were often observed (Fig. 2A, record at