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Small conductance chloride channels in the apical membrane of thyroid cells

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A small conductance chloride channel has been identified on the apical membrane of porcine thyroid cells using the patch-clamp technique. In cell attached membrane patches with NaCl in the pipette, the single channel conductance is 5.5 pS. The channel is highly selective for chloride over gluconate and iodide, and is impermeable to Na^+, K^+ and tetraethylammonium ions. The open state probability of the channel is not affected by voltage. The channel activity disappears after excision of the patch. The Cl^- channel blocker 5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB) did not affect the activity of the thyroid Cl^- channels. Treatment of thyroid cells with 8-(4-chlorophenylthio)adenosine-3',5'-cyclic monophosphate (8-chloro-cAMP) (0.5 mM) prior to giga-seal formation increased Cl^- channel activity in the apical membrane of thyroid cells.

Chloride channel; Thyroid cell; Patch-clamp

1. INTRODUCTION

Chloride channels in epithelia can regulate fluid secretion or electrolyte absorption by controlling the amount of chloride that is co-transported with its counter-ion. Cl^- channels have been characterized using patch clamp technique in a variety of fluid secreting epithelia. These Cl^- channels are responsible for the hormone-dependent Cl^- secretion [1]. Defective regulation of one class of secretory Cl^- channels seems to be a prominent characteristic of cystic fibrosis (e.g. [2]).

A number of data suggest the existence of passive Cl^- transport electrically coupled to the transport of Na^+ in culture thyroid cells [3,4].

The present work identifies a small conductance Cl^- selective channel in the apical membrane of thyroid cells in monolayer culture.

2. MATERIALS AND METHODS

2.1. *Cell cultures*

Porcine thyroid cells were isolated by a discontinuous trypsin-EGTA treatment [5]. Isolated cells were suspended in Eagle's minimum essential medium supplemented with non-essential amino acids, 10% new born calf serum, penicillin (50 U/ml) and streptomycin sulfate (50 μg/ml). 35 mm Falcon culture dishes were filled with 2 ml of cell suspension in a culture medium containing 2×10^6 cells/ml and incubated at 37°C in a 95% air/5% CO_2 water-saturated atmosphere. Monolayers reached confluency 3-4 days after plating and were routinely used after at least 6 days of culture. The medium was changed every 4 days.

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2.2. *Patch-clamp recordings*

The patch-clamp method [6] was applied to the apical membrane of thyroid cell in monolayer. Single channel currents were recorded from cell-attached membrane patches. Experiments were performed at 20°C. The resistance of the pipettes was 8-15 MΩ when filled with NaCl solution. Pipettes were coated with Sylgard resin to reduce current noise. The bath solution contained: 135 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM Hepes NaOH at pH 7.4. In most single channel experiments the pipette was filled with: 140 mM NaCl, 3 mM $MgCl_2$, 10 mM Hepes NaOH at pH 7.4. For KCl solutions, NaCl was replaced by an equal amount of KCl. The low Cl^- concentration solutions were obtained by replacing NaCl by 100 mM Na gluconate and 40 mM NaCl. Low Na^+ concentration solutions were obtained by replacing NaCl by 110 mM tetramethylammonium chloride (TMACl) and 30 mM NaCl. In NaI solutions, NaCl was replaced by an equal amount of NaI. In assays of stimulated channel activity, a bolus of 100 μl containing 5 mM 8-(4-chlorophenylthio)-adenosine 3',5'-cyclic monophosphate (8-chloro-cAMP; Sigma) was added to the bath (final concentration 0.5 mM). The chloride channel blocker, 5-nitro-2(3-phenylpropylamino) benzoic acid (NPPB) (Hoechst), was dissolved with dimethylsulfoxide (DMSO) (final concentration 0.1%).

Single channel currents were digitized at intervals between 2 and 10 ms by a digital oscilloscope (Nicolet Instrument Corp.) and stored on a hard disk using a Hewlett-Packard computer for further analysis. The corner frequency of the filter was 100 Hz. The channel amplitude (unitary current level) was determined by constructing amplitude histograms of the currents recorded at each potential. For kinetic analysis amplitude histograms were fitted by Gaussian curves, each curve reflecting a current level. The probability of being in any of the represented levels (P_n) was given by the fractional area and under each curve. The open-state probability of the channel was calculated from:

$$P_o = 1/N \sum_{n=1}^{N} n \cdot P_n$$

where N is the number of channels estimated from the number of current levels observed. A check was made by comparing the values

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measured for P_n with the values predicted from the binomial distribution of N channels with open probability P_o.

3. RESULTS AND DISCUSSION

When thyroid cells are cultured on a solid support, they form a well-polarized monolayer with the basolateral membrane facing the attachment substrate and the apical membrane facing the bath medium [7,8]. In the experiments described in this paper only the outer surface is accessible to he patch pipette.

Fig.1A shows a typical electrophysiological recording of a cell attached patch of the apical membrane of a thyroid cell with NaCl solution in the pipette. In this experiment two current levels of the same amplitude were seen, indicating that two channels were present in this membrane patch. The current-voltage relationship demonstrates that the single channel current is a linear function of the clamp voltage. Single channel currents reversed at a pipette potential close to 0 mV. Similar results were obtained in 6 different experiments with NaCl in the pipette. The mean single channel conductance was 5.5 ± 1.2 pS and the mean zero current pipette potential was 4 ± 2.5 mV. Since the channel activity of the channel disappeared within few seconds after excision of the patch (n = 9), the experiments reported in this paper were performed with cell-attached membrane patches. These results were obtained with control cells and when cells were stimulated before formation of the seal (see below).

Recordings were obtained with 140 mM KCl in the pipette are presented in fig.1B. Five similar experiments with KCl in the pipette gave a slope conductance of 5.2 ± 0.4 pS and a mean reversal potential of 1.5 ± 3.7 mV. The kinetic behaviour of the channel was characterized by long openings interrupted by long-lasting closing events of the order of sec and brief closing events. Fig.2B shows that the open-state probability of the channel does not vary with the applied potential. The fact that the conductive properties of the channel were not modified by replacement of NaCl in the pipette by KCl could mean either that the channel is non-selective for cations or that it selects for Cl^- over Na^+ and K^+.

However, anion substitution experiments indicate that we are dealing with an anion channel. Fig.1C illustrates one typical experiment in which the pipette was filled with 100 mM Na gluconate and 40 mM NaCl. In these conditions outflow of anions from the cell into the pipette could be observed at zero clamp voltage. The current events reverse their direction at pipette voltage exceeding −20 mV. The slope conductance is slightly decreased (4.9 pS) and the zero current pipette potential was −20 mV. Three experiments performed under the above conditions gave a mean slope conductance of 4.8 ± 0.1 pS and a mean zero current pipette potential of −22.5 ± 2.5 mV. Therefore, substituting the control NaCl solution for a low Cl^- solution in the pipette, shifted the zero current pipette potential from 4 mV to −23 mV. This change is close to the theoretical shift in E_{Cl} of −29 mV predicted by the Nernst equation, indicating that the current through this channel was carried by Cl^- ions.

The selectivity of the channel for Cl^- over Na^+ was confirmed in additional experiments in which a large part of NaCl in the pipette was replaced by TMACl (110 mM) (fig.1D). In 3 experiments the I/V curve was linear, the single channel conductance was 5.15 pS ± 0.2 pS and the channel current reversed at pipette potentials close to 0 mV (2.3 ± 4.3 mV). These results were similar to those obtained with NaCl or KCl solution in the pipette and confirmed that Cl^- is the conducting ion.

The permeability of the channel to iodide was investigated (fig.2A). When the pipette was filled with a NaI solution, outflow of anions from the cell into the pipette was recorded at positive pipette potentials whereas no detectable channel activity could be observed at negative pipette potentials. Therefore the anionic channel is impermeable to iodide. The I/V relationship confirms the conclusion.

It has been demonstrated that NPPB inhibits 50 pS in HT29 cells [9,10] and in the rectal gland of the dogfish [11] whereas it does not affect the 11 pS Cl^- channel in the dogfish rectal gland [15]. Fig.3A shows that the presence of 0.1 mM NPPB in the pipette solution has no significant effect on the Cl^- channel activity. Neither the kinetics (fig.2B), nor the current amplitude, were affected by the drug. Similar single channel current traces could be recorded over a period of 15 min after giga-seal formation. Identical observations have been made in a series of 5 experiments.

The activity of Cl^- channels is regulated by intracellular cAMP in fluid secreting epithelia [1]. On the other hand, TSH, adenylate cyclase activators and cAMP analogs have been shown to stimulate fluid transport in cultured thyroid cells [8,12]. We therefore investigated the possible effect of 8-chloro-cAMP on channel activity. When patches were quiescent, addition of 8-chloro-cAMP never caused the appearance of channel activity (n = 16) even after 30 min. When Cl^- channels were already active in control conditions, addition of 8-chloro-cAMP did not significantly affect channel activity and did not increase the number of active channels in the patch (n = 9) even after 30 min. However, Cl^- channel activity was observed more frequently when thyroid cells were treated prior to giga-seal formation. In these experiments, 100 μl of 8-chloro-cAMP (0.5 mM) was added to the bath and the length of the exposure varied between 5 min and 60 min. Fig.3B shows that when thyroid cells are treated before patching, the percentage of patches containing active Cl^- channels is increased compared to unstimulated cells from 20% to 50%. These results suggest

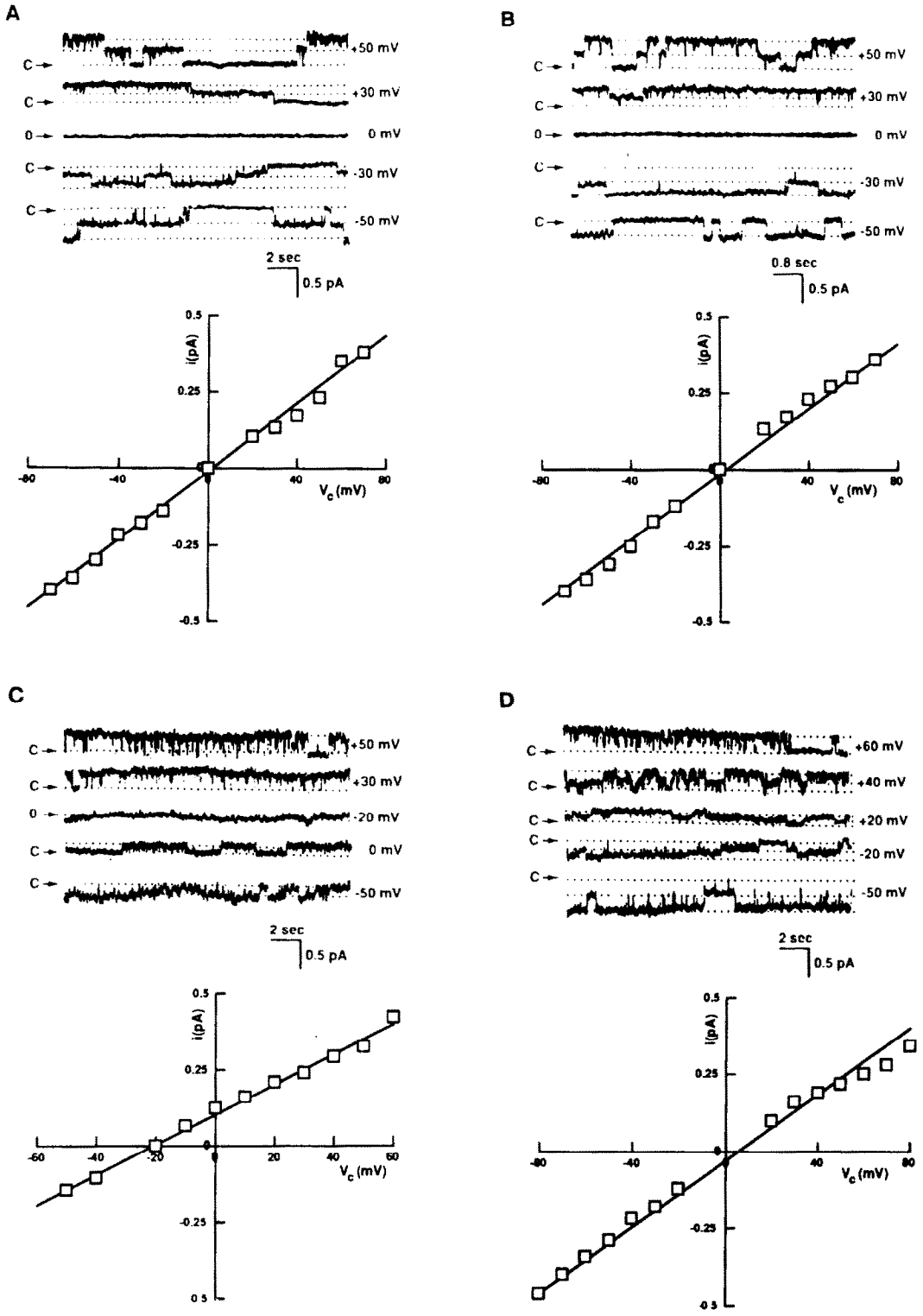

Fig.1. Single channel currents recorded at different pipette potentials from cell attached membrane patches and corresponding I/V relationships. The recording pipette was filled with NaCl solution (A), with KCl solution (B), with 100 mM Na gluconate and 40 mM NaCl (C) and with 110 TMACl and 30 mM NaCl (D). The sign of the applied potential refers to the bath with respect to the patch pipette interior. Applied potentials are noted on the right of the current traces. The zero pipette potential refers to the resting membrane potential. Upward currents refer to the flow of cations from the pipette into the cell or the flow of anions in the opposite direction. The baseline current with no channel open is indicated by the arrow marked C in each trace. The zero current level was indicated by O. Single channel current was plotted as a function of the pipette voltage. The data points were fitted by linear regression (solid line).

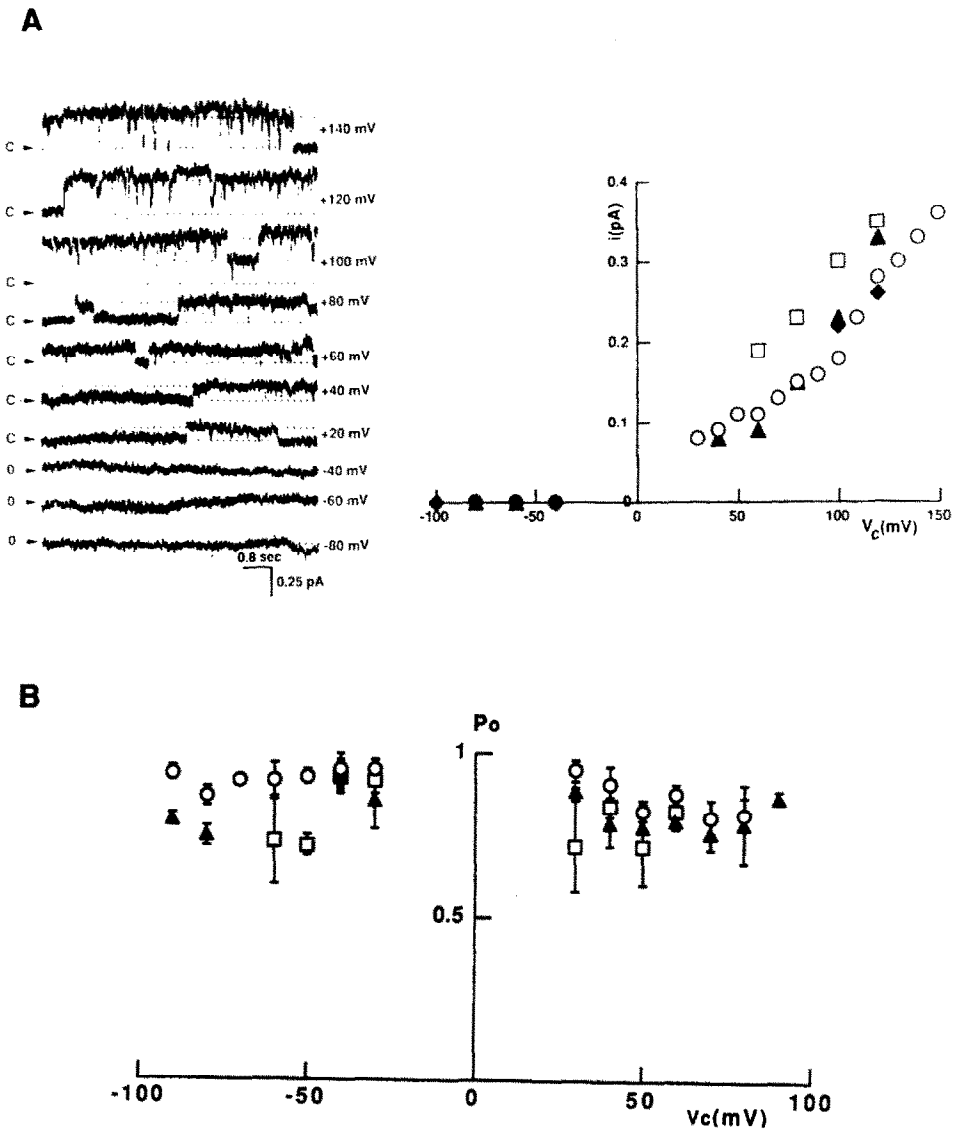

Fig.2.(A) Single channel recordings from a cell attached membrane patch with NaI in the pipette. The applied potentials are noted on the right. C indicates the closed state. O indicates the zero current level. (Right panel) Single channel current curves from the experiment shown in the left panel (○) and from other experiments obtained with different patches. (B) Relationship between mean values of open state probabilities (P_o) and clamp potentials V_c of Cl^- channels in cell attached membrane patches. The pipette was filled with NaCl (□), KCl (▲) and NaCl solution containing 0.1 mM NPPB (○). Error bars refer to the mean ± S.D. calculated from 3 different patches.

that stimulation may induce the incorporation of new channels into the apical membrane.

Cl^- channels could be tentatively classified in 3 main categories: (i) maxi Cl^- channels with a unit conductance of 200–450 pS which are blocked by SITS and DIDS; (ii) channels with an intermediate conductance of 50 pS which are blocked by NPPB, DPC analogues and stilbene disulfonate; (iii) channels with a lower unit conductance of 1–20 pS.

The third class of Cl^- channels appears to be very heterogeneous and the Cl^- channel described in this work show significant differences with other small conductance Cl^- channels. It is clearly different from voltage and Ca^{2+} dependent Cl^- channels which have been identified in endocrine cells from pars intermedia [13] and lacrimal glands [14] and from NPPB sensitive 15 pS Cl^- channels present in HT29 cells [10]. The channel under study shows some similarities with the 11 pS Cl^- channels in the dogfish rectal gland [15] such as the impermeability to I^- ions and the insensitivity to NPPB. However, 11 pS Cl^- channels in the dogfish rectal gland could only be observed in patches excised from cells pretreated with AMPc. The Cl^- channel described in this work could be similar to the 4 pS Cl^- channel recently identified in the pancreatic duct cells [16], for which there is unfortunately no pharmacological information.

The physiological role of the Cl^- channel described here is presently unknown. Since it has been already shown that cAMP is involved in the regulation of the

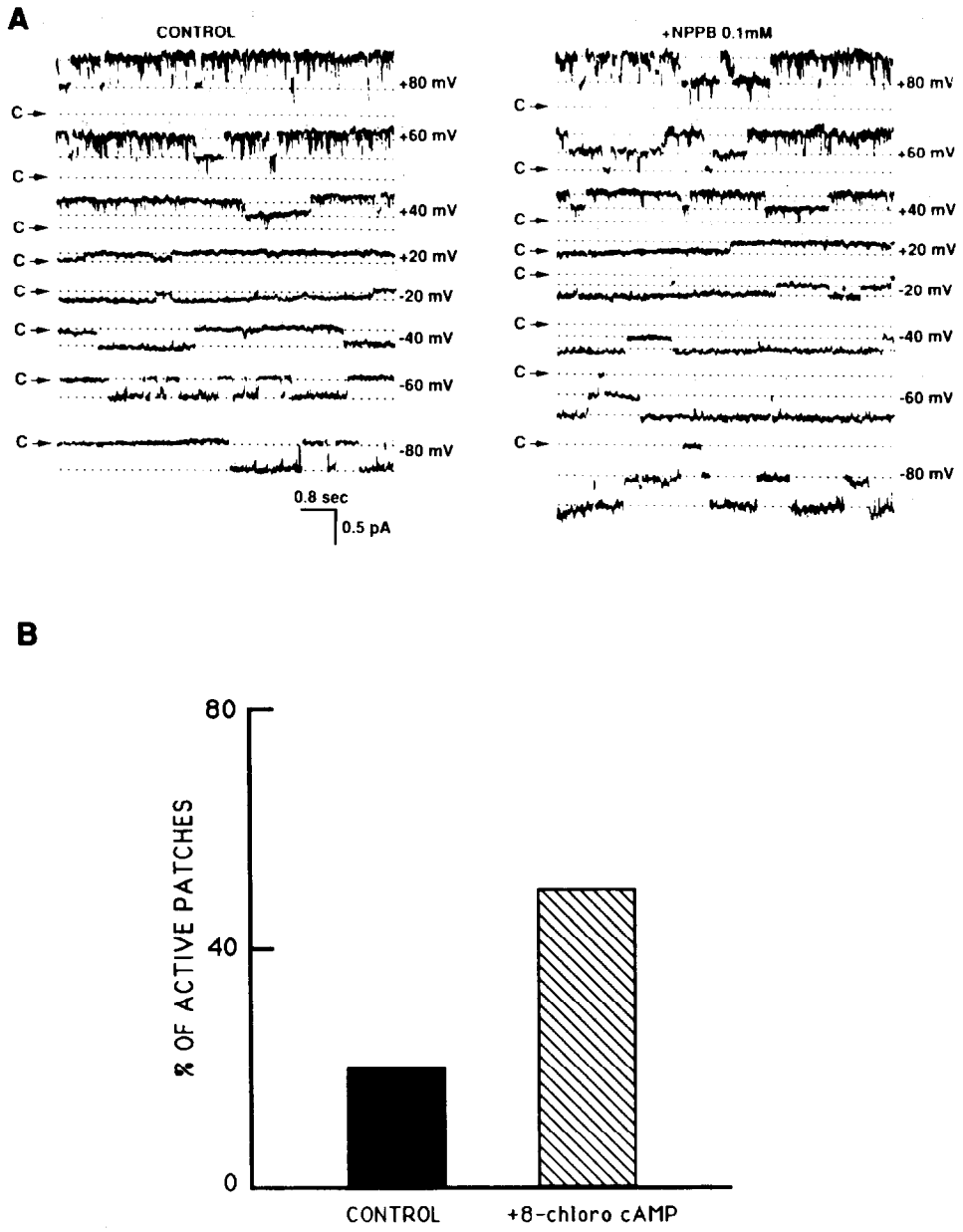

Fig.3.(A) Single channel currents from two different cell attached membrane patches with NaCl solution in the pipette, in the absence (left) and in the presence of 0.1 mM NPPB (right). (B) Effects of addition of 8-chloro-cAMP (0.5 mM) prior to giga-seal formation on the percentage of patches containing active Cl^- channels. The length of the exposure varied between 15 and 60 min. When cells were not pretreated, 15 patches among 78 contained active Cl^- channels. When cells were exposed to 8-chloro-cAMP prior to giga-seal formation, 53 out of 106 patches contained active Cl^- channels.

thyroid fluid transport system [8,12,17] and since a number of thyroid cell functions are TSH (i.e. cAMP) dependent [18], it is possible that these small Cl^- channels are involved, together with Na^+ permeable channels [19,20] in the modulation or control of the secretory activity.

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