Biochimica et Biophysica Acta 821 (1985) 229–232
Elsevier

BBA 72839

Calcium-activated cation channel in rat thyroid follicular cells

Y. Maruyama *, D. Moore and O.H. Petersen **

M.R.C. Secretory Control Research Group, Department of Physiology, University of Liverpool, P.O. Box 147, Liverpool, L69 3BX (U.K.)

(Received July 31st, 1985)

Key words: Cation channel; Ca^{2+} activation; Single-channel current; (Rat thyroid follicular cell)

Using the patch-clamp single-channel current recording technique, a cation channel in the contraluminal membrane of rat thyroid follicular cells has been characterized. The channel has a unit conductance of about 35 pS and is equally permeable to sodium and potassium. The pattern of channel opening and closing is independent of the membrane potential. The channel is only operational when the ionized calcium concentration in the fluid which is in contact with the inside of the membrane is at least 1 μM. This conductance pathway can be classified as a calcium dependent non-selective cation channel and could explain stimulant-evoked depolarizations in the thyroid follicular cells.

Introduction

Thyroid follicular cells have a high resting membrane potential of about −60 to −80 mV [1–4]. Thyroid-stimulating hormone has been reported to evoke membrane depolarization [4–7] and in a study on cultured rat thyroid cells, noradrenaline was shown to evoke membrane depolarization and resistance reduction and this effect was at least in part due to opening of a Na^+ conductance pathway mediated by intracellular Ca^{2+} [3]. The noradrenaline-evoked electrical changes in the cultured rat thyroid cells are very similar to those observed in mouse and rat pancreatic acinar cells in response to acetylcholine and various hormonal peptides [8,9]. In the mouse and rat pancreatic acinar cells single-channel current recording experiments have shown the presence of a non-selective monovalent cation channel that is activated directly by internal Ca^{2+} or indirectly by acetylcholine or cholecystokinin (via internal Ca^{2+}) [10–13]. The purpose of the investigation reported here was to find out whether similar channels were present in the thyroid gland. We now show directly the presence of Ca^{2+}-activated cation channels in the basal membrane of rat thyroid follicular cells.

Methods

Fragments of rat thyroid gland (about 50 mg) were digested with pure collagenase (150 U/ml) for 30 min in order to obtain isolated clusters of follicular cells similar to the procedure previously used to obtain isolated clusters of pancreatic acinar cells [10,11,12]. Single-channel currents were recorded with the patch-clamp method from membrane fragments excised from the basal cell surface in such a way that the physiological inside of the plasma membrane faced the bath and the outside the filling solution in the recording microelectrode (inside-out membrane patch) [14]. The standard 'extracellular' solution contained (mM): 140 NaCl, 4.7 KCl, 1.2 $CaCl_2$, 1.13 $MgCl_2$, 10 glucose, 10

* Present address: Department of Physiology, Faculty of Medicine, Tokyo University, Japan.
** To whom correspondence should be addressed.

Hepes (pH 7.2). The standard 'intracellular' solution contained (mM): 145 KCl, 10 NaCl, 1.13 $MgCl_2$, 10 glucose, 10 Hepes (pH 7.2). Different Ca^{2+} concentrations were used in the 'intracellular' solution and these are given in relation to the relevant experiments. Total Ca^{2+} concentrations were checked by atomic absorption spectroscopy. In experiments where ionized Ca^{2+} concentrations of 1 μM or below were needed Ca/EGTA buffers were used. All recordings were carried out at room temperature (about 22°C).

Results

When a membrane patch has been excised in the inside-out conformation and the inside is exposed to a solution containing a relatively high Ca^{2+} concentration ($[Ca^{2+}]_i = 100$ μM) clear unitary current steps can be observed at negative (normal) membrane potentials. Fig. 1 shows single-channel current traces from one patch at membrane potentials from -20 to -70 mV. The amplitude of the unitary steps increases with increasing polarization and there is a linear relation between single-channel current amplitude and membrane potential (Fig. 2) corresponding to a single-channel conductance of about 35 pS. When the membrane potential is changed from -30 mV to 0 (trace (a) in Fig. 1) the unitary steps disappear. Reversal of the membrane potential to $+30$ mV results in a pattern of fluctuating currents, but no clear unitary steps can be distinguished. The same results were obtained in three different ionic situations, i.e. quasi-physiological ion gradients (extracellular (Na^+) solution in pipette, intracellular (K^+) solution in bath) (three experiments), symmetrical extracellular solutions (five experiments) and symmetrical intracellular solutions (four experiments). Fig. 2 shows that the single-channel current-voltage relationship was identical in all three cases.

When Ca^{2+} was removed from the bath solution in contact with the inside of the excised patch membrane the unitary current steps disappeared. Fig. 3 shows a typical experiment in which $[Ca^{2+}]_i$ was changed from 100 μM to less than 1 nM and back again to 100 μM. The fully reversible closing of all channels induced by Ca^{2+} removal is clearly seen (five experiments). Fig. 4 shows a slightly

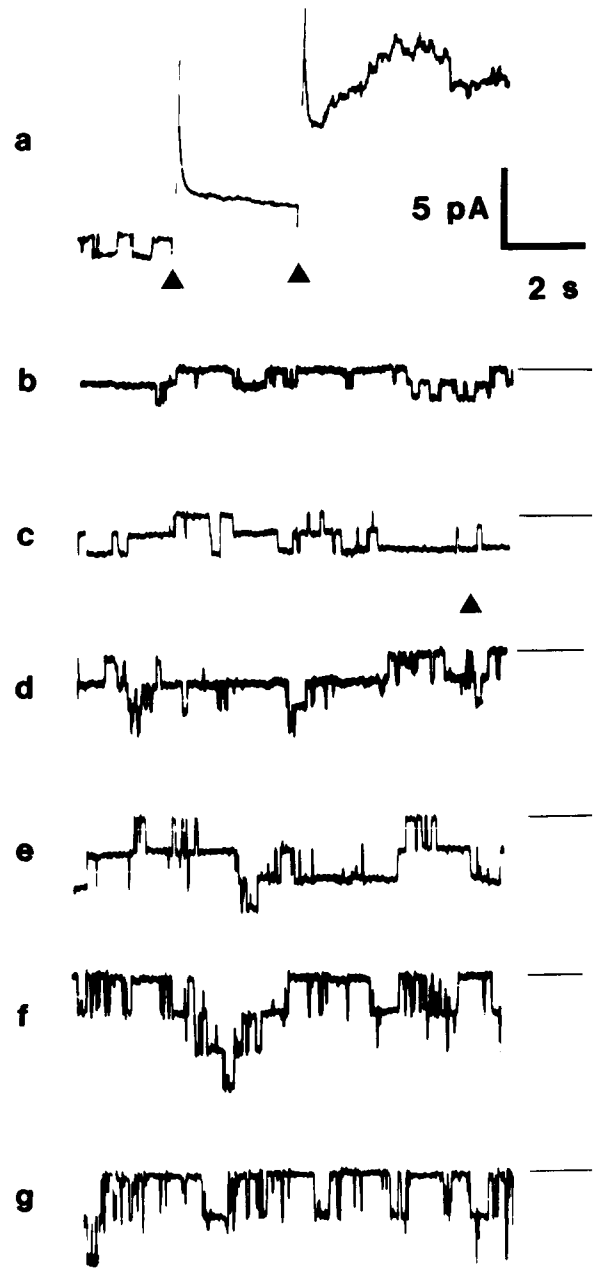

Fig. 1. Single-channel currents from excised inside-out membrane patch. Pipette was filled with extracellular solution whereas bath was filled with intracellular solution containing 100 μM Ca^{2+}. (a) Initially membrane potential was clamped at -30 mV but at first arrow it was changed to 0 mV. At second arrow membrane potential was reversed to $+30$ mV. (b) -20 mV, (c) -30 mV, (d) -40 mV, (e) -50 mV, (f) -60 mV and (g) -70 mV. Horizontal lines represent current level when all channels were closed.

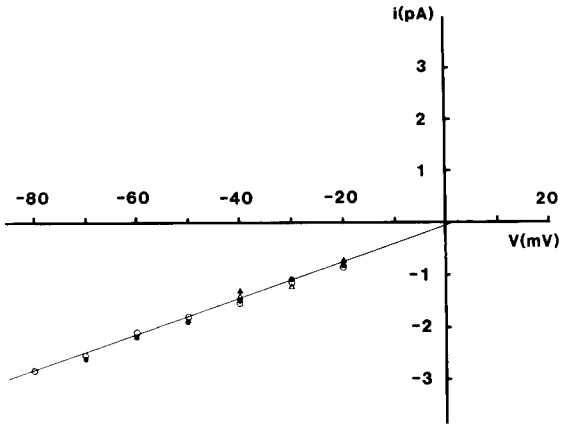

Fig. 2. Relationship between single-channel current and membrane potential in three different ionic situations. Open circles, extracellular solution in pipette, intracellular solution in bath (Na^+/K^+); closed circles, Na^+/Na^+; and triangles, K^+/K^+. In all cases $[Ca^{2+}]_i = 100\ \mu M$.

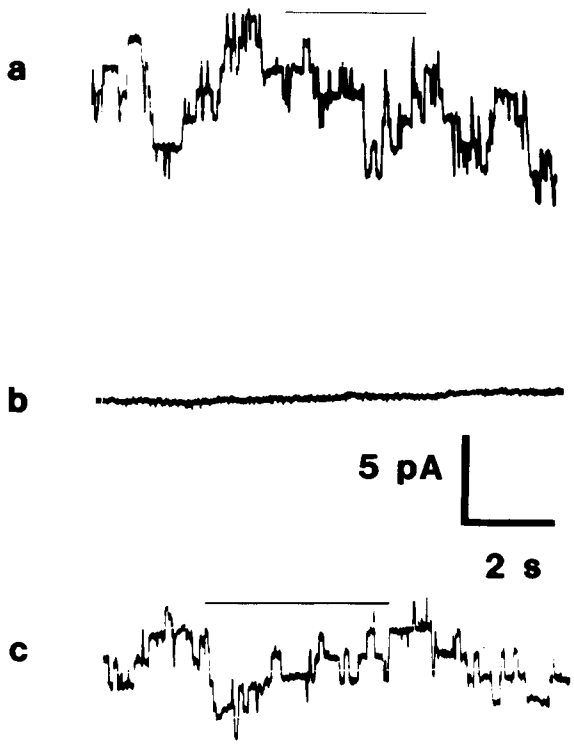

Fig. 3. The effect of removing Ca^{2+} from bath solution on single-channel currents. (a) was obtained with normal ionic gradients (Na^+/K^+) and $[Ca^{2+}]_i = 100\ \mu M$. (b) was obtained 1 min after replacement of bath solution with intracellular (K^+) solution without any Ca^{2+} added and with 1 mM EGTA ($[Ca^{2+}]_i < 1$ nM). (c) was obtained 1 min after return to control situation ($[Ca^{2+}]_i = 100\ \mu M$). Membrane potential was −40 mV throughout.

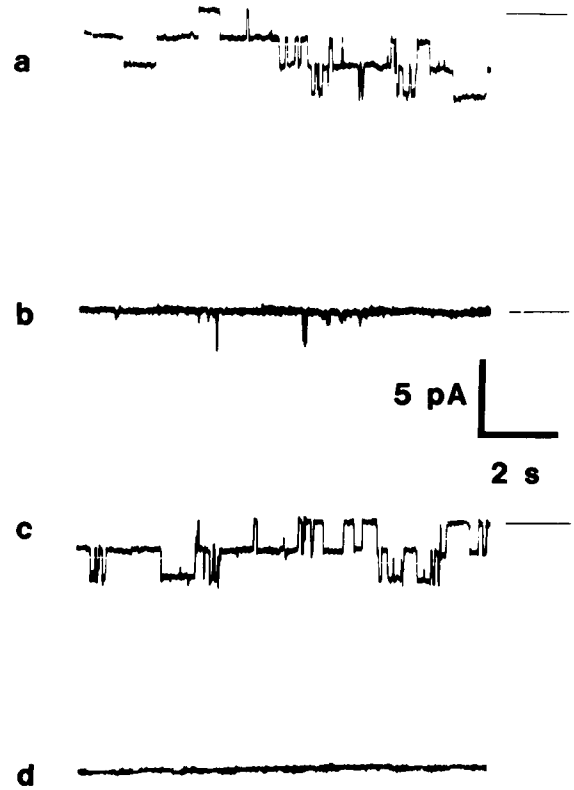

Fig. 4. The effect of changing $[Ca^{2+}]_i$ on single-channel currents. Same ionic situation as in Fig. 3. (a) $[Ca^{2+}]_i = 100\ \mu M$; (b) $[Ca^{2+}]_i = 1\ \mu M$ (total Ca concentration = 1 mM, total EGTA concentration = 1.1 mM); (c) $[Ca^{2+}]_i = 100\ \mu M$ and (d) $[Ca^{2+}]_i < 1$ nM (total Ca concentration is about 10 μM, total EGTA concentration is 1 mM). Membrane potential was −40 mV throughout.

more elaborate experiment in which $[Ca^{2+}]_i$ is first reduced from 100 μM to 1 μM evoking almost complete channel closure except for a few very brief openings. This effect is reversed after readmission of the intracellular solution with 100 μM Ca^{2+}. When thereafter all Ca^{2+} is removed ($[Ca^{2+}]_i < 1$ nM) all channel activity is abolished (three experiments).

Discussion

The pattern of single-channel current activity in excised membrane patches from rat thyroid follicular cells is remarkably similar to that described for mouse and rat pancreatic acinar cells [10–12]. The channel demonstrated in the thyroid cells (Figs. 1–4) has the same unit conductance (about

35 pS) and the same current-voltage relationship as in the mouse and rat pancreatic cells [10]. The channel fails to discriminate between Na^+ and K^+ and is activated by micromolar Ca^{2+} concentrations from the inside of the membrane as in the case of the pancreas [10,11]. The channel can therefore be unambiguously classified as the Ca^{2+}-activated non-selective cation channel. This channel was first found in cultured cardiac cells [15] and soon thereafter in neuroblastoma [16] and pancreatic cells [10]. The Ca^{2+}-activated non-selective cation channel is regarded as being voltage-insensitive [10,15,16] and this is confirmed here for the thyroid gland (Fig. 1). Over a wide range of negative membrane potentials (-20 to -70 mV) the pattern of channel opening and closure does not depend very markedly on the magnitude of membrane polarization. The lack of clear single-channel unitary steps at reversed (positive) membrane potential was unexpected and is in contrast to the results from the pancreatic cells [10–12] where the pattern of activity was the same at positive and negative membrane potentials.

The Ca^{2+}-activated non-selective cation channel shown here for the first time to be present in the contraluminal membrane of rat thyroid follicular cells could explain stimulant-evoked membrane depolarization [3–7] if it could be assumed that stimulation evoked an increase in $[Ca^{2+}]_i$. In the work of Sinback and Coon [3] it has indeed been shown that noradrenaline evokes depolarization and that intracellular Ca^{2+} injection mimicks this effect whereas intracellular injection of the Ca^{2+} chelator EGTA markedly reduces the noradrenaline-evoked response. Very similar findings have been made in mouse pancreatic acinar cells [17,18]. There is thus remarkable agreement between results obtained with intracellular microelectrodes in thyroid and pancreatic cells [3,17,18] as well as with patch-clamp single-channel current recording methods in these two tissues. TSH has also been reported to evoke membrane depolarization in several thyroid preparations [4–7] and since it has been shown that this hormone stimulates the efflux of Ca^{2+} from the cellular compartment [19] it seems possible that $[Ca^{2+}]_i$ is elevated in this condition and that activation of the non-selective cation channel therefore could take place explaining the reduction in membrane potential.

References

1 Green, S.T. and Petersen, O.H. (1981) Pflügers Arch. 391, 119–124
2 Green, S.T., Singh, J. and Petersen, O.H. (1982) Biochim. Biophys. Acta 720, 36–41
3 Sinback, S.N., and Coon, H.G. (1982) J. Cell Physiol. 112, 391–402
4 Takasu, N., Handa, Y., Shimizu, Y. and Yamada, T. (1985) Biochem. Biophys. Res. Commun. 129, 275–279
5 Woodbury, J.W. and Woodbury, D.M. (1963) J. Physiol. 169, 553–567
6 Williams, J.A. (1970) Endocrinology 86, 1154–1158
7 Pace, C.S. and Gaitan, E. (1979) Biochim. Biophys. Acta 555, 111–118
8 Petersen, O.H. (1980) The Electrophysiology of Gland Cells, Academic Press, London
9 Petersen, O.H. (1982) Biochim. Biophys. Acta 694, 163–184
10 Maruyama, Y. and Petersen, O.H. (1982) Nature 299, 159–161
11 Maruyama, Y. and Petersen, O.H. (1982) Nature 300, 61–63
12 Maruyama, Y. and Petersen, O.H. (1984) J. Membrane Biol. 81, 83–87
13 Petersen, O.H. and Maruyama, Y. (1984) Nature 307, 693–696
14 Hamill, O.P., Marty, A., Neher, E., Sakmann, B. and Sigworth, F.J. (1981) Pflügers Arch. 391, 85–100
15 Colquhoun, D., Neher, E., Reuter, H. and Stevens, C.F. (1981) Nature 294, 752–754
16 Yellen, G. (1982) Nature 296, 357–359
17 Iwatsuki, N. and Petersen, O.H. (1977) Nature 268, 147–149
18 Laugier, R. and Petersen, O.H. (1980) Pflügers Arch. 386, 147–152
19 Rodesch, F., Bogaert, Ch. and Dumont, J.E. (1976) Mol. Cell Endocrinol. 5, 303–313