Volume 303, number 2,3, 221–223 FEBS 11102 June 1992

Cytosolic chloride ions stimulate Ca^{2+}-induced exocytosis in melanotrophs

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Received 28 February 1992

We used the whole-cell patch-clamp technique to study the secretory activity of single cells by monitoring changes in membrane capacitance [Neher, E. and Marty, A. (1982) Proc. Natl. Acad. Sci. USA 79, 523–535] in anterior pituitary cells. Unexpectedly we have observed that increasing intracellular chloride ions stimulate Ca^{2+}-induced exocytosis in a dose-dependent fashion (K_d = 12 mM). These results demonstrate a role of cytosolic chloride ions in the regulation of exocytotic secretion in anterior pituitary cells. It is suggested that chloride channels, in addition to playing a part in regulating membrane electrical activity [Korn, S.J., Bolden, A. and Horn, A. (1991) J. Physiol. 439, 423–437; Penner, R., Matthews, G. and Horn, A. (1988) Nature 334, 499–504] and cytosolic pH [Kaila, K. and Voipio, J. (1987) Nature 330, 163–165], are also involved in the modulation of cytosolic chloride concentration and thus in the control of exocytosis.

Cytosolic chloride; Ca^{2+}-induced exocytosis; Patch clamp; Melanotroph; Rat; Membrane capacitance

1. INTRODUCTION

There have been contradictory results on the effects of chloride ions on the process of secretion in different tissues. Extracellular chloride ions were reported to be required for insulin secretion from pancreatic islet cells [5,6], epinephrine from adrenal chromaffin cells [7] and prolactin from normal and tumour pituitary cells [8,9], since replacement of chloride by isothionate resulted in an inhibition of secretion. In contrast to this it was reported that chloride ions are inhibitory to release of epinephrine to permeabilized adrenal chromaffin cells [10], and vasopressin from permeabilized neurohypophyseal nerve endings [11]. However, in the latter preparation low levels of chloride were stimulatory to vasopressin release [12]. In permeabilized mast cells it was shown that the presence of Cl^- affects the control mechanism of secretion [13].

To investigate the effect of cytosolic chloride ion concentration ($[Cl^-]_i$) on exocytotic secretion in a single rat melanotroph secreting a variety of peptides such as β-endorphin and melanocyte stimulating hormone [14], the whole-cell patch-clamp method was used. Since this technique allows the control of cytosol ionic composition [15] various amounts of Cl^- and Ca^{2+} were dialyzed into the cytosol, while the secretory activity indicated by changes in membrane capacitance (C_m) [3] was monitored. Our results show that high $[Cl^-]_i$ stimulates the Ca^{2+}-induced secretory activity in single anterior pituitary cells in a dose-dependent manner.

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2. MATERIALS AND METHODS

Cell culture from the rat pars intermedia region was prepared by a standard method [16] where only collagenase (Gibco) and deoxyribonuclease (Sigma) were used for enzymatic disintegration. Instead of fetal calf serum and chick embryo extract, serum replacement Ultroser G (3–6%; Gibco) was used. Cell-covered poly-L-lysine-treated coverslips were kept in the incubator at 36°C, 95% humidity and in a 5% CO_2 atmosphere for 1–14 days before experimentation. Then the coverslips were transferred into the recording chamber mounted onto an inverted microscope (IM 35) in the recording medium (in mM): NaCl 131.8; KCl 5; $MgCl_2$ 2; $NaHPO_4$ 0.5; $NaHCO_3$ 5; HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid)/NaOH 10; D-glucose 10; $CaCl_2$ 1.8; pH 7.2) and viewed with a X40 phase-contrast objective.

The cells were voltage-clamped [17] at a holding potential of −70 mV. Membrane capacitance (C_m) was recorded using a two-phase lock-in amplifier (1600 Hz, 1 mV peak-peak) incorporated into a patch-clamp amplifier [18]. The plots of the passive cell parameters, access conductance (G_a), parallel combination of leak and membrane conductance (G_m) and C_m, were derived by a computer-aided reconstruction using an analogue-to-digital converter (CED 1401, Cambridge, UK) to record the signals in digital format on the computer hard disk (IBM compatible). The DC current (low pass, 1–10 Hz, −3 dB), holding potential and the real and imaginary admittance signals (low pass 1 Hz, −3 dB) were used in the calculation. The software was written by Dr. John Dempster (University of Strathclyde) and utilized reversal potential for calculating the passive parameters [19]. Recordings were made at room temperature with pipette resistances between 2 to 4 Ω (measured in potassium gluconate solution), giving access conductances of more than 150 nS (in potassium gluconate solutions). The pipette solution contained (in mM): $MgCl_2$ 2; HEPES 10; EGTA (ethyleneglycol-bis-(aminoethyl ether)-*N'N'N'N'*-tetraacetic acid) 0.5 and Ca^{2+}-saturated EGTA 3.5 ($[Ca^{2+}]_i$ around 1 μM) or EGTA 4 ($[Ca^{2+}]_i$ around 30 nM [20]; Na_2ATP 2; potassium gluconate 150; pH 7.2 (KOH). In order to prepare various chloride concentrations KCl-

replaced potassium gluconate, or KCl- and potassium gluconate-containing pipette solutions were mixed. These pipette solutions were of similar osmolarity (within 5%) measured by freezing point depression (Camlab, Cambridge, UK). EGTA and Ca^{2+}-saturated EGTA were prepared as 100 mM stock solutions as described previously [21]. Intracellular $[Ca^{2+}]_i$ was estimated assuming an apparent dissociation constant for the Ca–EGTA complex of 0.15 μM [22]. All salts were obtained from Sigma.

3. RESULTS AND DISCUSSION

In accordance with already published results [20,23] cytosol dialysis with high Ca^{2+} ($[Ca^{2+}]_i$ = 1 μM) resulted in an increase in C_m, which exceeded that recorded at low $[Ca^{2+}]_i$ (only EGTA in the pipette; Fig. 1A; see also Fig. 2A). In both cells displayed in Fig. 1A cytosolic $[Cl^-]$ was 4 mM. At increased $[Cl^-]_i$ (154 mM) Ca^{2+}-induced increase in C_m, relative to the resting C_m, was about 2-times larger (Fig. 2B). The maximum rate of C_m rise increased by about 4-fold (Fig. 2A).

Changes in C_m reflect the balance between exocytosis and endocytosis, and the larger Ca^{2+}-induced increase in C_m at 154 mM $[Cl^-]_i$ is consistent with an increased rate of exocytosis. It is possible that such an increase in C_m could be due to a Cl^--dependent decrease in rate of endocytosis and an unchanged rate of exocytosis. However, this event is unlikely to occur since the rate of endocytosis at low $[Ca^{2+}]_i$ also appeared to be increased in the presence of high cytosolic Cl^- (154 mM), which is indicated by the small decline in C_m (compare bottom signals in Fig. 1; see Fig. 2).

The secretory response of single anterior pituitary cells to cytosol dialysis with 1 μM $[Ca^{2+}]_i$ clearly depends on $[Cl^-]_i$, with a half-maximal stimulation at around 12 mM (Fig. 3). This suggests an effect of Cl^- on exocytotic machinery. The site of action of Cl^- is not known, but structures like G-proteins may be a target since they were shown to modulate exocytosis in anterior pituitary cells [24] and Cl^- was found to exert modulatory effects on GTP-dependent regulatory proteins [25].

The intracellular $[Cl^-]$ is not known in anterior pituitary cells but levels of about 40–50 mM were measured in a clonal ACTH-secreting pituitary cell line [2]. It is likely that Ca^{2+}-sensitive Cl^- channels, described in normal [26] and clonal anterior pituitary cells [27], may be involved in mediating changes in $[Cl^-]_i$. It can be estimated that a sustained Cl^- current of 1 pA can significantly change $[Cl^-]_i$ over a period of a few minutes in these small cells. The passive influx of Cl^- would be enhanced since membrane potential in these cells appears to be around −40 mV [28,29]. Consistent with this, when extracellular chloride $[Cl^-]_o$ was replaced by isothionate 30–60 min before the experiment the secretory response (measured as the rate of C_m increase) to cytosol dialysis with 1 μM $[Ca^{2+}]$ and 4 mM $[Cl^-]$ was further reduced from 4.8 ± 0.9 to 3.1 ± 0.7 fF/s (n=13, P=0.14, Student's t-test). This suggests that subplasmalemmal $[Cl^-]_i$ at 160 mM $[Cl^-]_o$ is higher than the concentration dialyzed into the cytosol by the pipette (4 mM).

In summary, our results demonstrate a modulatory role of $[Cl^-]_i$ on Ca^{2+}-induced secretory activity of single anterior pituitary cells. It is suggested that Ca^{2+}-sensitive chloride channels found in the membrane of anterior pituitary cells, in addition to regulating membrane excitability [2] and cytosolic pH [4], may play a role in modulating $[Cl^-]_i$ and thus controlling the Ca^{2+}-sensitive exocytotic hormone secretion.

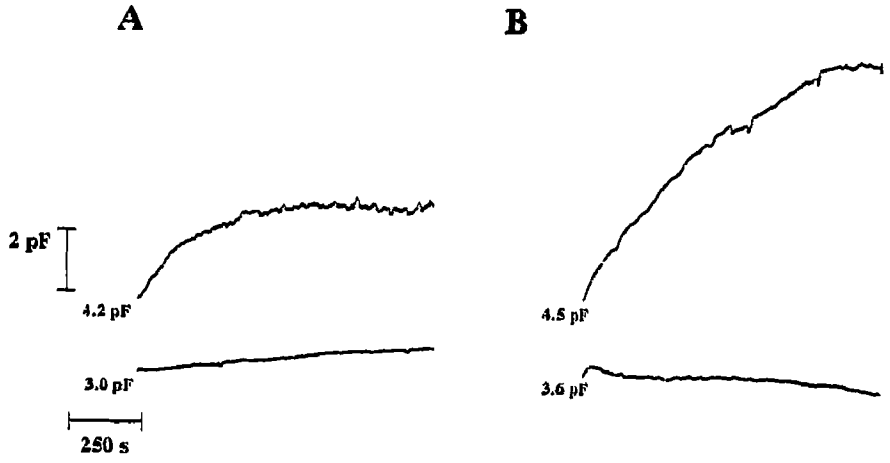

Fig. 1. Representative membrane capacitance changes indicating secretory responses of rat melanotrophs at different ionic conditions. (A) Cytosolic concentration of Cl^- was 4 mM. The upper trace represents a response to cytosol dialysis with 1 μM $[Ca^{2+}]_i$. The maximal rate of C_m increase was 5 fF/s; after 10 min stationary level was reached which was 30% larger than the resting C_m. The lower trace shows a response where $[Ca^{2+}]_i$ was low (≈30 nM [20] with only EGTA in the pipette). (B) Pipette-filling solution contained 154 mM of Cl^-. The top trace shows a response in C_m with 1 μM $[Ca^{2+}]_i$ in the pipette. The maximal rate of C_m increase was 15 fF/s, and an apparent saturation level of 80% above resting C_m was measured after about 15 min of cytosol dialysis. At low $[Ca^{2+}]_i$ (lower trace) the response to dialysis with 154 mM $[Cl^-]_i$ resulted in a small decline of C_m with a rate of 3 fF/s. Numbers adjacent to traces indicate resting membrane capacitance, which was subtracted.

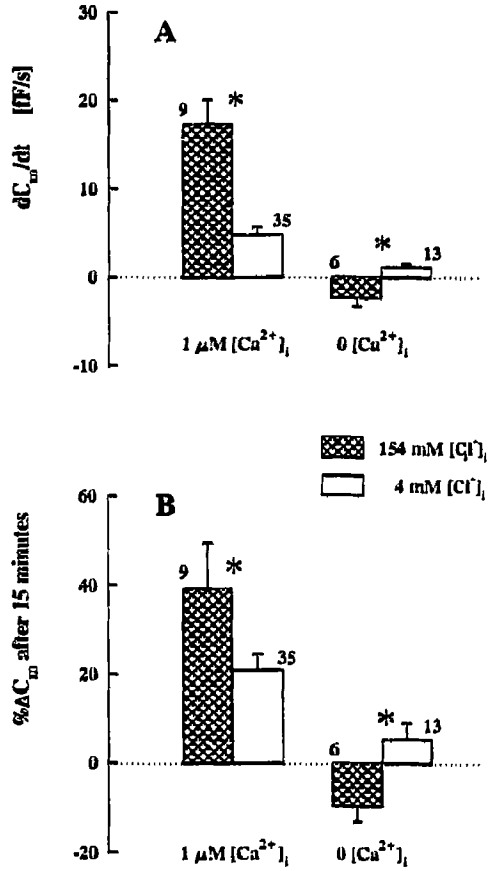

Fig. 2. Bar chart representation of the secretory response (indicated by changes in C_m) of rat melanotrophs to cytosol dialysis with low (4 mM) and high (154 mM) $[Cl^-]$ at high (around 1 μM) or low (around 30 nM) $[Ca^{2+}]_i$. (A) The maximal rate of secretion is different (statistically significant differences are denoted by an asterisk, $P<0.01$) at both concentrations of cytosolic chloride at high $[Ca^{2+}]_i$. Similarly we noticed differences in rates of C_m changes at nominally Ca^{2+}-free pipette solutions. (B) Relative changes in membrane capacitance following 15 min of cytosol dialysis. Vertical bars indicate S.E.M.; numbers adjacent to columns indicate numbers of cells tested.

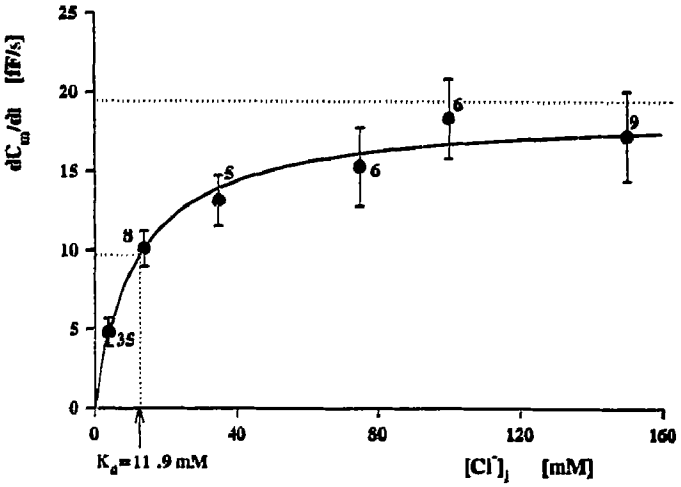

Fig. 3. The effect of different intracellular chloride concentrations on the maximal rate of increase in C_m recorded in single rat melanotrophs. The dissociation constant (K_d) was determined by fitting the data to the following equation: $dC_m/dt=(dC_m/dt)_{max.} \times [Cl^-]_i/(K_d+[Cl^-]_i)$. The asymptote for the maximal rate of C_m increase was 18.7 ± 2.6 fF/s and K_d 11.9 ± 0.4 mM Cl^- (see arrow and dotted lines). Symbols represent the mean; error bars represent standard error of the mean. Numbers near the symbols show numbers of cells tested.

Acknowledgements: This work was supported by the Ministry of Science and Technology of Slovenia, Grant P3-0155-381. We would like to thank Professor M. Kordaš for support.

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