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Effects of Cl^- deficiency on the membrane potential in mouse pancreatic β-cells

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The membrane potential of mouse pancreatic β-cells was measured with microelectrodes. In the resting cell (3 mM D-glucose), the membrane potential was -63 ± 3 mV (mean ± S.E. for four experiments). In the presence of 3 mM D-glucose, total Cl^- substitution by isethionate induced a depolarization by 3–4 mV, and readmission of Cl^- induced a hyperpolarization by 3–5 mV. At 10 mM glucose, reduction of Cl^- to 12 mM by substituting isethionate for Cl^- reversibly shifted the repolarization potential by 6–9 mV in the positive direction and stimulated the burst activity during the initial 2–3 min by increasing the fraction of plateau phase. This was followed by a gradual inhibition of electrical activity, including decrease in fraction of plateau phase and slow wave amplitude. Total substitution of Cl^- by isethionate or methyl sulphate reversibly shifted the repolarization potential by 3–4 mV in the positive direction and rapidly inhibited the electrical burst pattern without any initial stimulation. Glucose-induced (10 mM) insulin release (15 min) and $^{45}Ca^{2+}$ uptake (3 min) were strongly inhibited by reducing the Cl^- concentration to 10 mM (isethionate as substitute) and were further inhibited by further reduction of the Cl^- concentration. It is suggested that β-cells are equipped with on electrogenic Cl^- flux, which can affect the burst pattern of electrical activity. The inhibitory effects of Cl^- substitution may be explained by an influence of Cl^- on the voltage-controlled Ca^{2+} channels.

Introduction

Glucose-induced insulin release is inhibited by Cl^- substitution by the less permeable anions isethionate [1–4], methyl sulphate [4], sulphate [3] or *p*-aminohippurate [5]. This is probably not due to inhibition of glucose metabolism in the pancreatic β-cells, as the production of $^{14}CO_2$ from ^{14}C-labelled glucose is only slightly affected by Cl^- substitution [2,6]. Studies on $^{36}Cl^-$ transport in pancreatic islets have shown that glucose stimulation is paralleled by a marked increase in the rate of $^{36}Cl^-$ efflux across the β-cell plasma membrane [1,7]. Such stimulation also produces a net reduction in the $^{36}Cl^-$ equilibrium content in the islet cells [1]. These data raised the question of whether glucose-induced Cl^- efflux from the β-cells may be electrogenic and participate in the electrical activity induced by glucose [1,5].

The aim of the present study was to test whether β-cells possess mechanisms for Cl^- electrodiffusion, by measuring the effects of Cl^- substitution by less permeable anions on the β-cell membrane potential and electrical activity using intracellular microelectrodes.

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Materials and Methods

Animals and preparation of pancreatic islets

Studies of the β-cell membrane potential were performed with semidissected islets from adult NMRI mice. For studies of insulin release and $^{45}Ca^{2+}$ uptake, microdissected islets from non-inbred, adult *ob/ob*-mice (Umeå *ob/ob*) were used. These mice have hyperplastic pancreatic islets, which are particularly rich in β-cells (greater than 90%; [8]) that show normal responses to stimulators and inhibitors of insulin secretion [9]. The large amounts of islet tissue and high proportion of β-cells make these islets particularly suitable for studies of β-cell function.

Measurements of membrane potential

The membrane potential of single β-cells was recorded with microelectrodes, according to the methods previously described in detail [10]. In brief, microelectrodes with a tip resistance greater than 200 MΩ were impaled into β-cells in islets whose surface was exposed by gentle dissection of pieces of pancreas. The β-cells were identified by their typical glucose-induced burst pattern of electrical activity and its dependence on the glucose concentration [11]. The preparation was continuously perifused with medium (see below) supplemented with glucose and with ionic modifications as required. Membrane potential was continuously recorded on tape and monitored on an ink recorder (W&W 1100) and on an oscilloscope. The figures shown were obtained by playback of the tape to a fast ink recorder (Brush Accuchart, Gould Inc.).

The incubation medium used in the studies of membrane potential had the following composition in mM: NaCl, 135; KCl, 4.7; $MgCl_2$, 1.2; $CaCl_2$, 2.6; and Hepes, 10. The medium was adjusted to pH 7.40 with NaOH and gassed with 100% O_2. Cl^- deficiency was obtained by replacing NaCl by equimolar sodium isethionate or sodium methyl sulphate (partial Cl^- substitution) and in some experiments in addition replacing KCl by potassium acetate, $MgCl_2$ by $MgSO_4$, and $CaCl_2$ by calcium acetate (total Cl^- substitution). Control experiments showed that the addition of 9.9 mM acetate to normal medium did not change the membrane potential or electrical activity of β-cells. All experiments on membrane potential were performed in the Department of Physiology I, University of Saarland.

Measurements of insulin release and $^{45}Ca^{2+}$ uptake

Insulin release was measured in static incubations of microdissected pancreatic islets. Batches of three islets were first preincubated for 30 min at 37° C in 1 ml basal medium (see below) followed by 15 min of incubation in the same type of medium supplemented with glucose and with ionic modifications as required. Insulin released into the incubation medium was measured by radioimmunoassay, using mouse insulin as standard. The uptake of $^{45}Ca^{2+}$ was measured with the La^{3+}-wash technique, as previously described [12].

In experiments on insulin release and $^{45}Ca^{2+}$ uptake, the incubation medium had the following composition in mM: NaCl, 130; KCl, 4.7; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $CaCl_2$, 2.6; and Hepes, 20. This medium was adjusted to pH 7.40 with NaOH and equilibrated with ambient air. The secretory response or $^{45}Ca^{2+}$ uptake were not significantly changed by gassing the medium with 100% O_2. Cl^- deficiency was obtained by replacing NaCl by equimolar sodium isethionate and, in some experimental groups, in addition replacing KCl by potassium acetate and/or $CaCl_2$ by calcium acetate. Control experiments showed that the addition of 1–10 mM acetate to normal medium did not affect $^{45}Ca^{2+}$ uptake or insulin release. In studies of insulin release, the media were supplemented with 1 mg bovine serum albumin/ml as insulin carrier. Previous studies have shown that substitution of Cl^- by isethionate induces only a modest decrease in the Ca^{2+} activity [5]. All measurements of insulin secretion and $^{45}Ca^{2+}$ uptake were performed in the Department of Histology and Cell Biology, University of Umeå.

Chemicals

$^{45}Ca^{2+}$ was from Amersham International (U.K.) and ^{125}I-insulin from Hoechst AG (Frankfurt/Main, F.R.G.) Bovine serum albumin (fraction V) was obtained from Miles Laboratories (Stoke Poges) (U.K.), sodium isethionate (sodium 2-hydroxyethanesulphonate) from Sigma Chemical Co. (MO, U.S.A.) and sodium methyl sulphate from Merck-Schuchardt (München, F.R.G.). Other

chemicals were commercially available reagents of analytical grade.

Results

Control experiments with the tip of the measuring electrode placed in the perifusion medium just outside the pancreas indicated that the substitution of Cl^- by isethionate affects the measuring system (Fig. 1A). Total Cl^- replacement with isethionate as principal substitute caused a rapid change in the potential in the negative direction (8.3 ± 0.50 mV, $n = 12$) and readmission of Cl^- caused a corresponding shift in the positive direction (8.1 ± 0.54 mV, $n = 12$). These shifts were not changed by leaving 12 mM Cl^- in the medium or changing the glucose concentration from 3 to 10 or 20 mM (data not shown). Similar effects of Cl^- substitution have previously been observed in other systems (cf. Ref. 13) and are probably due to effects of the changed anion composition on the liquid-junction potential at the reference electrode. The data in Fig. 1A were used for correction of membrane potential measurements.

Also the resting membrane potential of β-cells in the presence of 3 mM glucose was shifted in the negative direction by total Cl^- deficiency (isethionate) and in the positive direction when Cl^- was reintroduced (Fig. 1B) after correction for the change in liquid-junction potential as depicted in Fig. 1A, it became evident that Cl^- deficiency produced a depolarization by 3–4 mV and that readmission of Cl^- caused a hyperpolarization by 3–5 mV over the test periods (Fig. 1C). These data

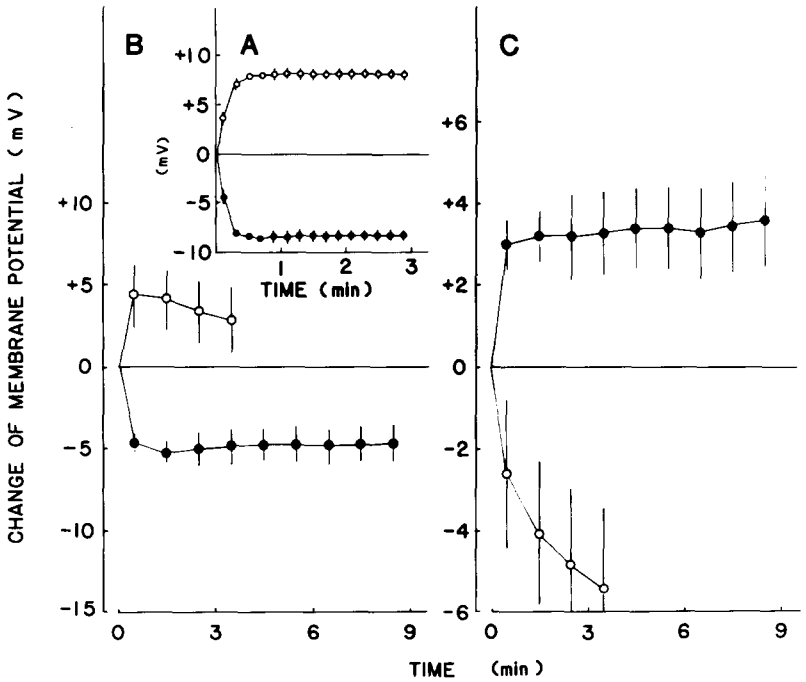


Fig. 1. Effects of substituting Cl^- by isethionate on resting membrane potential of β-cells at 3 mM glucose. (A) Recordings with the tip of the measuring electrode in solution just outside the pancreas. The lower curve (●) represents total removal of Cl^- and addition of isethionate as principal substitute (see Materials and Methods) and the upper curve (○) represents readmission of Cl^-. Measurements every 12 s were made from the ink recordings. (B) Impalements of β-cells; the lower curve (●) represents total replacement of Cl^- with isethionate as principal substitute and the upper curve (○) readmission of Cl^-. Measurements every 12 s were made from the ink recordings and the data were used for calculation of mean values for 1 min periods. (C) The data from panel B have been corrected for the shift in liquid-junction potential (panel A) during the corresponding time period. Upper curve (●) represents substitution of Cl^- and lower curve (○) readmission of Cl^-. Correction for dead space corresponding to 15 s has been made in A–C. Values represent means ± S.E. for 12 (panel A), 4 (filled circles in panel B and C) and 3 (open circles in panel B and C) separate experiments.

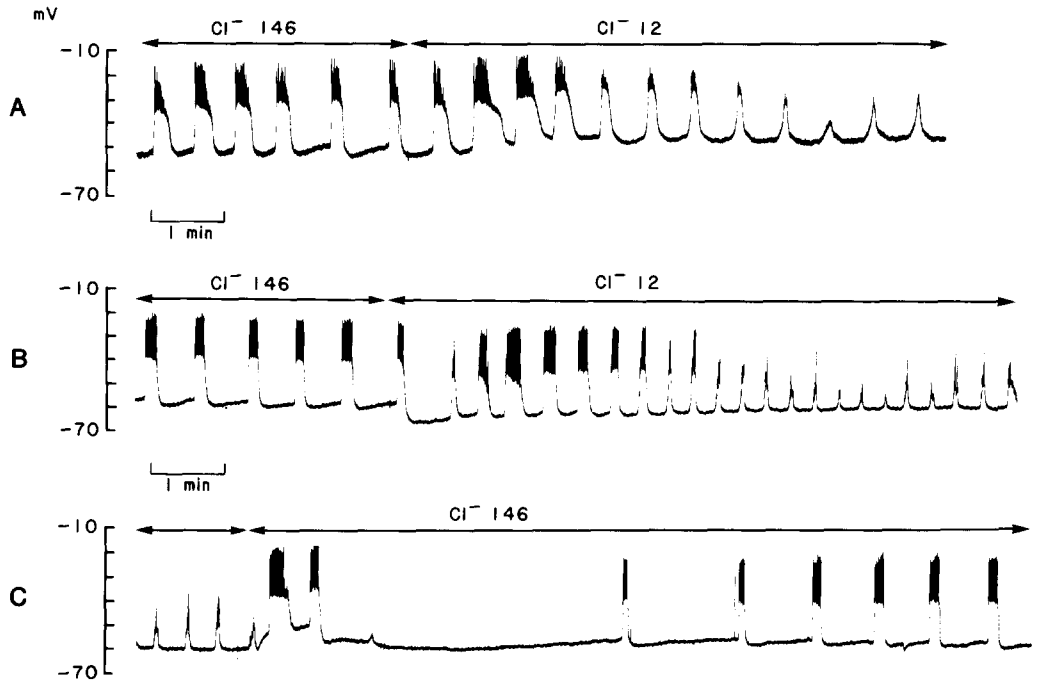
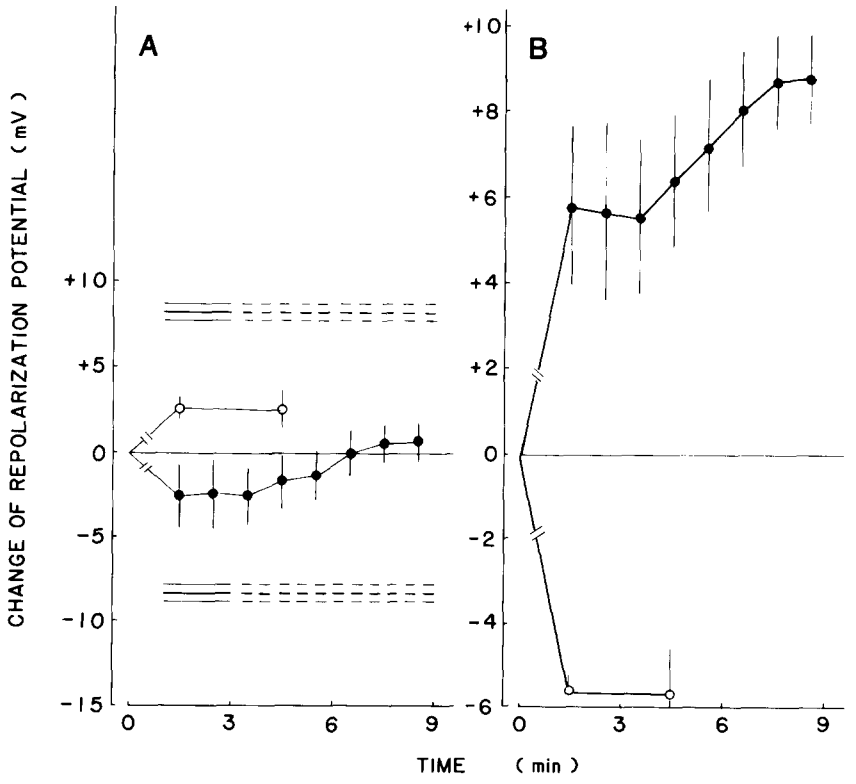


Fig. 2. Effect of partial Cl^- substitution by isethionate on membrane potential of single β-cells at 10 mM glucose. (A) and (B) represent recordings of two separate β-cells from different animals. Cl^- was substituted by isethionate leaving 12 mM Cl^- in the medium. (C) is a direct continuation of (B). Time for new medium to reach the β-cell corresponds to 15 s and has not been corrected for the figure.



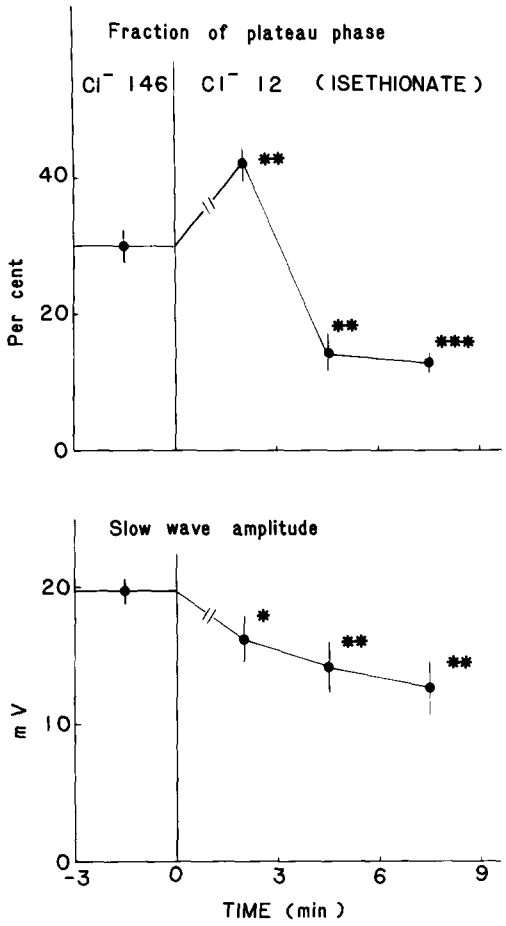


Fig. 4. Effect of partial Cl^- substitution by isethionate on fraction of plateau phase and slow wave amplitude at 10 mM glucuse. The data represent the same experiments as those shown in Fig. 3. Fraction of plateau phase is defined as the fraction of time spent at depolarized plateau with spiking and slow wave amplitude was measured as the difference between repolarization potential (see Fig. 3) and depolarized plateau in each cycle. The means ± S.E. for sever separate experiments were calculated over periods of 3 min, excluding the first min after Cl^- substitution (see legends to Fig. 3). * $P < 0.05$, ** $P < 0.02$ and *** $P < 0.001$ for difference from mean values during min −3 to 0.

TABLE I

EFFECT OF SUBSTITUTION OF Cl^- BY ISETHIONATE ON INSULIN RELEASE

After a preliminary incubation for 30 min in the presence of 3 mM D-glucose (period 1), isolated pancreatic islets were incubated for 15 min (period 2) in basal medium containing 3 or 10 mM D-glucose and with Cl^- substitution as indicated. Values represent means ± S.E. for the numbers of separate experiments given in parentheses.

[Cl^-] (mM)		[Glucose] (mM)	Insulin release (ng/μg dry islet per 15 min)
period 1 (30 min)	period 2 (15 min)		
139.8	139.8	3 (control)	0.029 ± 0.006 (11)
139.8	9.8	3	0.014 ± 0.005 (11)
139.8	4.7	3	0.020 ± 0.009 (10)
139.8	0	3	0.032 ± 0.017 (11)
139.8	139.8	10 (control)	0.411 ± 0.060 [a] (11)
139.8	9.8	10	0.130 ± 0.017 * (11)
139.8	4.7	10	0.077 ± 0.016 * (9)
139.8	0	10	0.074 ± 0.016 * (9)

[a] $P < 0.001$ for difference between 0.411 and 0.029 and * $P < 0.001$ for difference from the 10 mM glucose control using Student's t-test for paired data.

show that acute omission or readmission of extracellular Cl^- influence the membrane potential of the β-cell. D-Glucose at 10 mM evoked a typical electrical activity consisting of repetitive slow waves on which a fast spike activity is superimposed, as previously described in detail [10,11]. Replacement of most of the Cl^- by isethionate, leaving 12 mM Cl^- in the extracellular medium, induced several changes in the pattern of electrical activity. As illustrated by two representative β-cells from two different animals (Fig. 2), after a short period allowing for equilibration of the new medium in the perfusion chamber, the Cl^--poor medium caused a stimulation of the electrical burst pattern during 2–3 min. This was followed by a gradual inhibition of the burst pattern. A more

Fig. 3. Effect of partial Cl^- substitution by isethionate (12 mM Cl^- remaining in the medium) on repolarization potential at 10 mM glucose. (A) The lower curve (●) shows change in repolarization potential between the slow waves after substituting Cl^-. The data were calculated as mean values over 1 min periods. Data for the first minute after Cl^- substitution were not included because the transition between the two media caused a very variable pattern during this minute. The data were calculated as change from the mean value of repolarization potential during min −3 to 0 in each experiment. The upper curve (○) represents readmission of Cl^- calculated on a 3 min basis. The horizontal lines represent means ± S.E. of the liquid-junction potential shift, shown in Fig. 1A. (B) The data from panel (A) have been corrected for change in liquid-junction potential. Upper curve (●) represents partial substitution of Cl^- and lower curve (○) readmission of Cl^-. Values denote means ± S.E. for seven (●) and four (○) separate experiments.

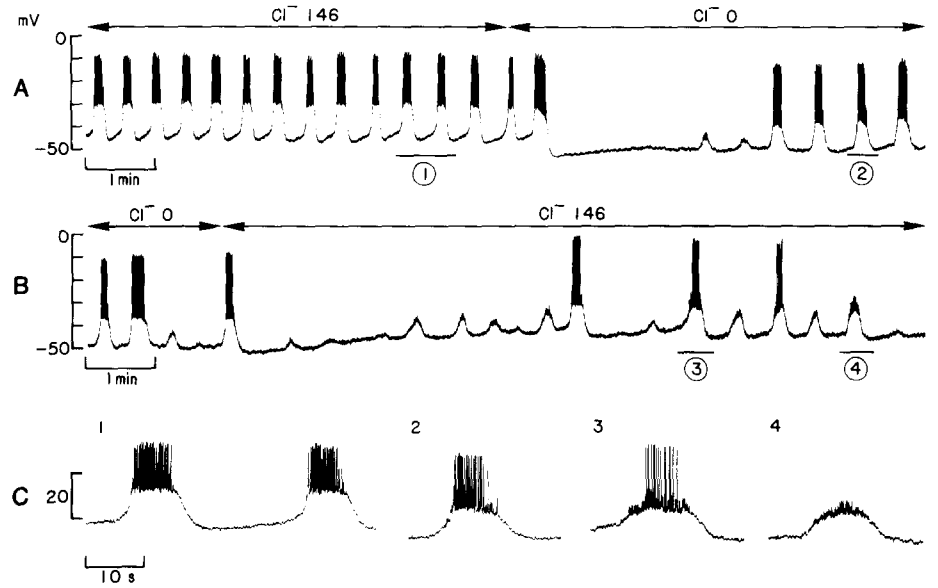


Fig. 5. Effect of total Cl^- substitution with isethionate as principal substitute on membrane potential of single β-cell at 10 mM glucose. In this and the following figure, traces 1–4 show portions of the electrical pattern with an expanded time scale and with a higher voltage gain. Trace B is a direct continuation of trace A. Dead space of 15 s has not been corrected for. These records are representative of results obtained from 12 different animals.

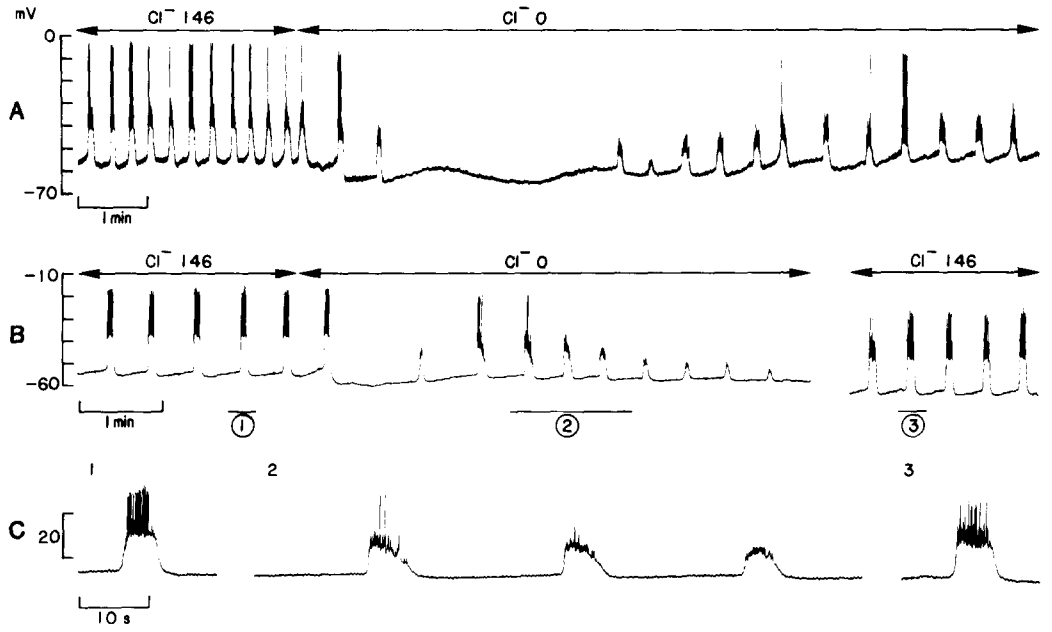


Fig. 6. Effect of total Cl^- substitution with methyl sulphate as principal substitute on membrane potential of single β-cells at 10 mM glucose. (A) and (B) represent two single β-cells from different mice. The record of recovery of burst pattern in (B) starts 15 min after readmission of Cl^- (not shown), following a total of 15 min of Cl^- deficiency, of which the last 10 min is not shown. Traces 1–3 are parts of the electrical pattern as described in Fig. 5. These traces are representative of results obtained in six different animals.

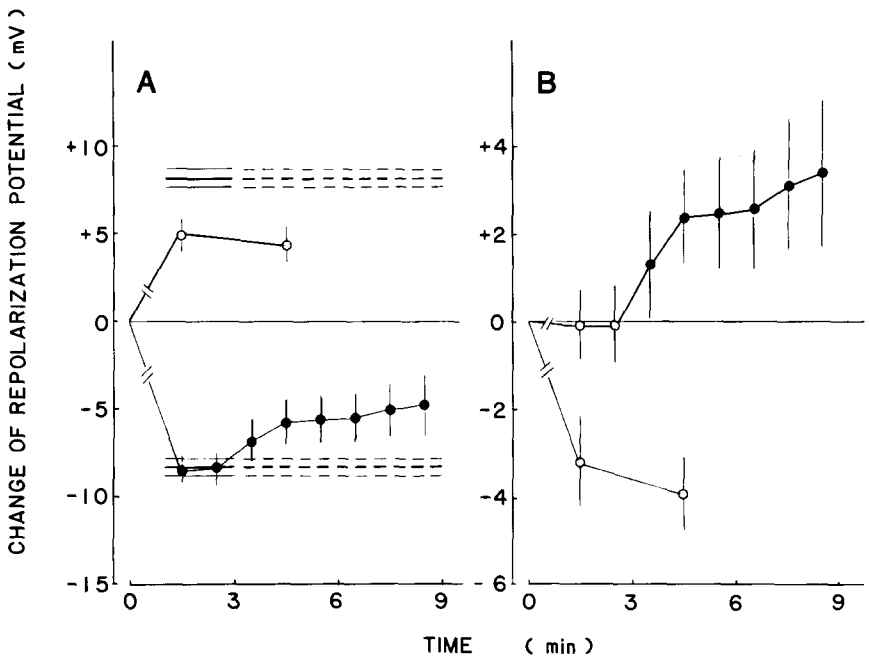


Fig. 7. Effect of total Cl^- substitution with isethionate as principal substitute on repolarization potential at 10 mM glucose. Changes in repolarization potential were calculated as described in Fig. 3 (see legend for description of (A), (B)). Values denote means ± S.E. for 12 (●) and 10 (○) separate experiments.

detailed analysis of these changes (Fig. 3A) showed that switching from normal to Cl^--poor medium produced changes in the repolarization potential that were much smaller than the liquid junction potential shown in Fig. 1A. After correction for this junction potential shift, the change in repolarization potential caused by Cl^- deficiency amounted to plus 6–9 mV, and the change in repolarization potential caused by readmission of Cl^- was approx. −6 mV.

Fig. 4 shows the effects of partial Cl^- substitution by isethionate on the fraction of plateau phase with spiking and slow wave amplitude. The fraction of plateau phase increased from 30.2 ± 2.2% (mean ± S.E. for min −3 to min 0) to 42.1 ± 2.4% (min 1 to min 3) ($P < 0.01$), followed by a

TABLE II

EFFECT OF SUBSTITUTION OF Cl^- BY ISETHIONATE ON $^{45}Ca^{2+}$ UPTAKE

After two successive periods of preliminary incubations in nonradioactive medium (periods 1 and 2), isolated pancreatic islets were incubated for 3 min (period 3) in basal medium supplemented with $^{45}Ca^{2+}$ (0.3 TBq/mol) and containing 3 or 10 mM D-glucose and with Cl^- substitution as indicated. The Cl^- substitutions also applied to period 2 and the D-glucose concentration during period 1 was 3 mM in all groups. The values represent means ± S.E. for the numbers of experiments indicated in parentheses.

[Cl^-] (mM)			[Glucose] (mM) in periods 2 and 3	$^{45}Ca^{2+}$ uptake (pmol/μg dry islet per 3 min)
period 1 (18 min)	period 2 (12 min)	period 3 (3 min)		
139.8	139.8	139.8	3	2.21 ± 0.18 (13)
139.8	139.8	139.8	10 (control)	3.74 ± 0.33 [a] (13)
139.8	9.8	9.8	10	2.72 ± 0.21 * (12)
139.8	4.7	4.7	10	2.64 ± 0.16 * (12)
139.8	0	0	10	2.38 ± 0.16 * (13)

[a] $P < 0.001$ for difference between 3.74 and 2.21 and * $P < 0.02$ for difference from control using Student's t-test for paired data.

marked decrease during min 3–9. The slow wave amplitude decreased gradually over the first 9 min after switching to Cl^--poor medium.

Total Cl^- substitution with isethionate (Fig. 5) or methyl sulphate (Fig. 6) as principal substitutes resulted in a rapid inhibition of the electrical burst pattern, without any initial stimulatory phase. Both types of anion substitution produced essentially the same changes, suggesting that the results represent the effect of extracellular Cl^- deficiency. After several minutes with Cl^--deficient medium, most β-cells spontaneously showed some degree of restitution of the electrical burst pattern. However, these slow waves usually had a reduced amplitude and showed only rudimentary spike activity (Fig. 5C and 6C). After readmission of Cl^-, most β-cells recovered a regular burst pattern. However, in cells with some albeit distorted, burst activity left at the time of adding Cl^-, the reintroduction of Cl^- led to a rapid inhibition of this activity and only after several minutes did a recovery of the regular activity occur (Fig. 2 and 5).

Fig. 7A shows an analysis of the changes in repolarization potential after total Cl^- replacement with isethionate as principal substitute. After correction for the change in liquid-junction potential shown in Fig. 1A, it became clear that, after a lag period of about 3 min, the repolarization potential was shifted in the positive direction by 3–4 mV and readmission of Cl^- induced a shift in the negative direction by about 4 mV (Fig 7B). Thus, the changes in repolarization potential were clearly less pronounced when Cl^- was totally substituted (Fig. 7B) than after leaving 12 mM Cl^- (Fig. 3B).

Further quantification of the changes in the slow wave system after total Cl^- substitution (Fig. 8) indicated that both the fraction of plateau phase and slow wave amplitude were rapidly decreased. After 3 min there was some tendency to an increase in the fraction of plateau phase (compare with Fig.s 5 and 6).

Substitution of Cl^- by isethionate produced a strong inhibition of glucose-stimulated (10 mM D-glucose) insulin release during incubations for 15 min (Table I), whereas the basal insulin release (3 mM D-glucose) was not affected (Table I). Such Cl^- substitution also strongly decreased $^{45}Ca^{2+}$

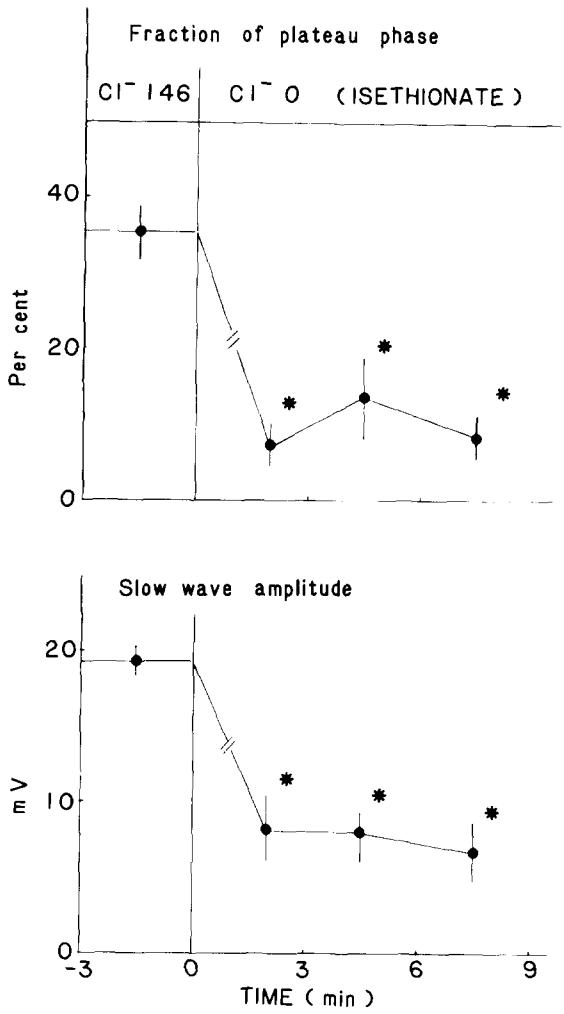


Fig. 8. Effect of total Cl^- substitution with isethionate as principal substitute on fraction of plateau phase and slow wave amplitude at 10 mM glucose. The data represent the same experiments as those shown in Fig. 7. The means ± S.E. for 12 experiments were calculated as described in the legend to Fig. 4. * $P < 0.001$ for difference from mean value during min −3 to 0.

uptake during incubations for 3 min, after 12 min of pretreatment with Cl^- deficient medium (Table II). Cl^- substitution by isethionate did not change the basal $^{45}Ca^{2+}$ uptake in the presence of 3 mM D-glucose (data not shown). Both insulin release and $^{45}Ca^{2+}$ uptake were markedly inhibited when Cl^- was reduced to 9.8 mM (71% and 66%, respectively) and both processes showed further decreases, which were dose-dependent, when the residual Cl^- concentration was reduced from 9.8 to

4.7 or 0 mM by substituting acetate for Cl^- (Tables I and II).

Discussion

Information about the exact role of chloride ions in the regulation of membrane potential in the pancreatic β-cells is scanty. Dean and Matthews [14] concluded that the reduction in extracellular Cl^- concentration (Cl_0^-) from 115 to 12 mM did no affect the β-cell membrane potential, and glucose still induced electrical activity. In a more recent study [15], it was shown that reduction in Cl_0^- from 127 to 7.5 mM by isethionate substitution produced a slight depolarization of the β-cells. Total omission of Cl_0^- caused, in addition to the slight depolarization, an inhibition of glucose-induced electrical burst activity. The authors considered these data consistent with a passive distribution of Cl^- and suggested that the depolarization induced by Cl^- substitution was due to redistribution of Cl^- and K^+ to re-establish Donnan equilibrium. It was speculated that the inhibition of glucose-induced electrical activity observed after total Cl^- substitution may be mediated by change in pH_i due to inhibition of Cl^-–HCO_3^- exchange [15].

The present results indicate that replacement of Cl^- by the less permeable anions, isethionate or methyl sulphate, produces distinct changes in the β-cell membrane potential. Several lines of evidence suggest that the β-cell membrane is equipped with Cl^- conductance. Firstly, in agreement with previous findings [15], acute omission of Cl_0^- produced a depolarization of the resting β-cell membrane and a reduction of the repolarization potential in glucose-stimulated β-cells. These changes were reversed upon readmission of Cl^-. Secondly, reduction of Cl_0^- from 146 to 12 mM stimulated the glucose-induced electrical burst pattern during the initial 3 min by increasing the fraction of plateau phase with spiking. This occurred in parallel with a positive shift in the repolarization potential by about 6 mV. After readmission of Cl^-, there was a hyperpolarization and inhibition of the burst pattern. Thirdly, previous studies have shown that the β-cell membrane is highly permeable to $^{36}Cl^-$ [1,7] and that only a fraction of this flux is inhibited by HCO_3^- deficiency or the addition of 4-acetamido-4′-isothiocyanostilbene-2,2′-disulphonic acid (SITS), a blocker of anion exchange [1,5]. This would speak against the hypothesis [15] that Cl^- substitution affects the β-cell electrical activity mainly by change in pH_i due to impaired Cl^-–HCO_3^- exchange. Fourthly, the previous suggestion [15] that depolarization produced by substitution of extracellular Cl^- is caused by net outflow of K^+ is contradicted by the observations that such Cl^- substitution in fact inhibits $^{86}Rb^+$ (K^+ analogue) efflux from prelabelled β-cells [4,6] and does not reduce the intracellular concentration of $^{86}Rb^+$ at isotope equilibrium (Lindström, Norlund, Sandström and Sehlin, unpublished data). These lines of evidence suggest that substitution of extracellular Cl^- by less permeable anions induces an electrogenic efflux of Cl^- that depolarizes the β-cell membrane and, by this depolarization, under certain conditions stimulates the regular burst pattern induced by glucose.

However, the present data also indicate that Cl^- substitution affects the β-cell in a more complex manner. When 12 mM Cl^- was left in the solution, the initial stimulation of electrical activity gradually changed into inhibition and at total Cl^- substitution only inhibition could be observed. The inhibition was characterized by both reduced fraction of plateau phase and reduced amplitude of the slow waves. Previous data suggest that the depolarization phase of the slow waves may be due to Ca^{2+} influx into the β-cell [16]. The inhibition of electrical activity by Cl^- substitution is therefore compatible with the inhibition of glucose-induced $^{45}Ca^{2+}$ influx, as shown here and in previous reports [4,6]. It is thought that glucose-induced $^{45}Ca^{2+}$ influx to a large extent represents flux through voltage-dependent Ca^{2+} channels (for review, see Ref. 17). Taken together, these results would suggest that Cl^- can directly or indirectly influence the voltage-controlled Ca^{2+} influx into the β-cells. As previously pointed out [18], this could reflect an anion selectivity of the Ca^{2+} channel rather than a strict Cl^- dependence.

Based on the present results, the role of Cl^- in the β-cell membrane potential and insulin secretion can be summarized in a model that comprises two basic mechanisms: Cl^- permeability and an anion-sensitive Ca^{2+} channel. The complex net

effects of Cl^- substitution, as shown in the present data, may be explained at least partly by the fact that these two basic mechanisms would mediate opposite electrical responses to a decrease in Cl_0^-. The electrodiffusion would tend to depolarize the membrane and stimulate an existing burst pattern because of the outwardly directed electrochemical potential gradient, whereas inhibition of Ca^{2+} channels would inhibit the burst pattern, which is to a significant extent based on Ca^{2+} current. In each experimental situation the result of Cl^- substitution may reflect the balance between the two effects. That an initial stimulation of the electrical activity was seen after partial Cl^- substitution but not after total substitution may be due to the concentration dependence of the Ca^{2+} channel on Cl^- in the low concentration range. About 34% of the glucose-induced $^{45}Ca^{2+}$ influx remained when Cl_0^- was reduced to about 10 mM, whereas only 12% of the influx remained after total Cl^- substitution. This effective block may override any stimulatory effect of Cl^- current.

The observation that the repolarization potential between the slow waves showed a more pronounced positive shift in the presence of 12 mM Cl^- than after total Cl^- substitution is not easy to interpret. It appears not to be due to the acetate added, because control experiments showed no effect of millimolar concentrations of acetate added to the normal medium. It could be speculated that a Cl^- conducting channel in the β-cell requires a critical concentration of Cl^- on the outer side of the membrane. Such trans-side ion dependence has previously been suggested for K^+ channels [19,20].

In conclusion, the present results suggest that β-cells are equipped with electrogenic Cl^- flux, which can to some extent contribute to the glucose-induced electrical burst pattern. It is also suggested that the voltage-controlled Ca^{2+} channels in the β-cells shown Cl^- sensitivity that can be explained in terms of either strict Cl^- dependence or anion selectivity.

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