Biochimica et Biophysica Acta, 982 (1989) 62–72
Elsevier

BBAMEM 74444

Ca^{2+}-activated K^+ channel in rat pancreatic islet B cells: permeation, gating and blockade by cations

Joseph A Tabcharani * and Stanley Misler

Departments of Medicine (Jewish Hospital) and Cell Biology / Physiology and the Program in Biomedical Engineering, Washington University, St Louis, MO (U S A)

(Received 18 November 1988)

Key words Potassium ion channel, Divalent cation activation, Voltage activation, Hydrogen ion activation, Cation selectivity, Pancreatic islet cell

Activation of Ca^{2+}-dependent K^+ conductance has long been postulated to contribute to the cyclical pauses in glucose-induced electrical activity of pancreatic islet B cells Here we have examined the gating, permeation and blockade by cations of a large-conductance, Ca^{2+}-activated K^+ channel in these cells. This channel shares many features with BK (or maxi-K^+) Ca^{2+}-activated K^+ channels in other cells. (1) Its 'permeability' selectivity sequence is $P_{Tl^+} \cdot P_{K^+} \cdot P_{Rb^+} \cdot P_{NH_4^+} \cdot P_{Na^+,Li^+,Cs^+} = 1.3.1\,0\ 0\,5{:}0.17{:} < 0.05$. Permeant, as well as impermeant, cations reduce channel conductance (2) Its conductance saturates at 325–350 pS with bath KCl > 400 mM (144 mM KCl pipette). (3) It shows asymmetric blockade by tetraethylammonium ion (TEA) and Na^+. (4) It is sensitive to Ca_i^{2+} over the range 5 nM–100 μM; over the range 50–200 nM, channel activity varies as $[Ca^{2+}\ free]^{1-2}$. (5) It is sensitive to internal pH over the range 6 85–7.35, but the decrease in channel activity seen with reduced pH_i may be partially compensated by the increase in free Ca^{2+} concentration which occurs on acidification of buffered Ca^{2+}/EGTA solutions

Introduction

Since the discovery that injection or trapping of Ca^{2+} in cells produces transient hyperpolarization and increased membrane potassium permeability [1], activation of Ca^{2+}-dependent potassium (or $K^+(Ca^{2+})$) channels in cell plasma membranes has been proposed to underlie a variety of cellular phenomena These include the periodic pauses of electrical activity displayed by neurons and endocrine cells which fire as bursting pacemakers, as well as receptor-mediated changes in potassium permeability (P_{K^+}) in cells which display changes in cytosolic Ca^{2+} [2] Since the identification of single $K^+(Ca^{2+})$ channels by patch-clamping [3–5], the biophysical study of their gating and ionic permeability has become an area of intense activity Some of these channels have unexpectedly large conductances, are exquisitely sensitive to Ca^{2+}, and are gated as well by membrane voltage (for a review, see Blatz and Magleby Ref 6)

Rodent pancreatic islet B cells are bursting pacemaker cells set in action by enhanced metabolism of fuel substrates An early model of B-cell excitability proposed that closure of the $K^+(Ca^{2+})$ channels underlies the slow glucose-induced decline in P_{K^+} and depolarization which then triggers repetitive trains of Ca^{2+}-dependent action potentials Re-opening of the $K^+(Ca^{2+})$ channels, due to Ca^{2+} accumulation during the spike train, was considered to underlie the periods of electrical quiescence separating spike trains [7] More recently, it has been found that many cell-attached patches of rodent B cells contain large-conductance channels which open and carry outward current in response to large depolarizations, often beyond the range of the action potential (e g , Refs 8 and 9) In inside-out excised membrane patches, these channels are clearly identified as large-conductance, $K^+(Ca^{2+})$ channels whose probability of opening is enhanced by nanomolar increases in bath Ca^{2+} (e g , Refs 10 and 11)

During the course of investigating the activity of the maxi-conductance $K^+(Ca^{2+})$ channel for its possible physiological role [12], we had the opportunity to ex-

* Present address Department of Physiology, McGill University, Montreal, Quebec, Canada H3G 1Y6

Abbreviations EGTA, ethyleneglycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid, IS, intracellular-like solution, ES, extracellular-like solution, TEA, tetraethylammonium ion, NBA, *N*-bromoacetamide

Correspondence S Misler, Renal Division (Yalem 713), The Jewish Hospital of St. Louis, 216 S Kingshighway Boulevard, St. Louis MO 63110 U S A

amine, in excised patches, its interaction with cations which permeate, block or gate it These results demonstrate that the large conductance $K^+(Ca^{2+})$ channel of the B cell is fundamentally similar to the 'maxi', or BK, $K^+(Ca^{2+})$ found in many other cells Some of these results have previously been represented in abstract form [13,14]

Materials and Methods

The general methods for islet preparation and culture and basic patch-clamp recording and channel analysis were identical to those we have previously described [15] Using pipettes filled with intracellular-like solution (IS), defined below, cell-attached patched were formed on the largest identifiable cells in small islet cell clumps exposed to 0 glucose extracellular-like solution (ES) The bath solution was rapidly changed to IS, thus abolished the membrane potential of the cell Patches which seemed promising for study displayed two types of unitary channel current, as shown in Fig 1a (1) A 60–65 pS voltage-independent channel was seen at all membrane potentials, it is the ATP-sensitive K^+, or K^+ (ATP), channel characteristic of B cells (2) A 200–250 pS voltage-dependent channel was seen during large steady-state membrane depolarizations ($V_c > +100$ mV with respect to bath ground), this is a calcium- and voltage-activated K^+, or $K^+(Ca^{2+})$, channel These patches were excised in either the inside-out or outside-out patch configuration In approx 80% of excised patches, K^+ (ATP) channel activity rapidly disappeared [15], but $K^+(Ca^{2+})$ channel activity persisted and was stable during steady-state depolarization (Figs 1b, 1 and 2) Patches which displayed time-dependent $K^+(Ca^{2+})$ channel inactivation, 'cycling' of channel activity through long, closed periods or sudden high-frequency bursts of activity (Figs 1b, 3 and 4), were discarded During each experiment, the $K^+(Ca^{2+})$ channel was demonstrated to be Ca_i^{2+}-activated by raising bath Ca^{2+} to 0 5 mM and noting the greatly enhanced activity In the outside-out patch, the $K^+(Ca^{2+})$ channel was recognized as a large conduc-

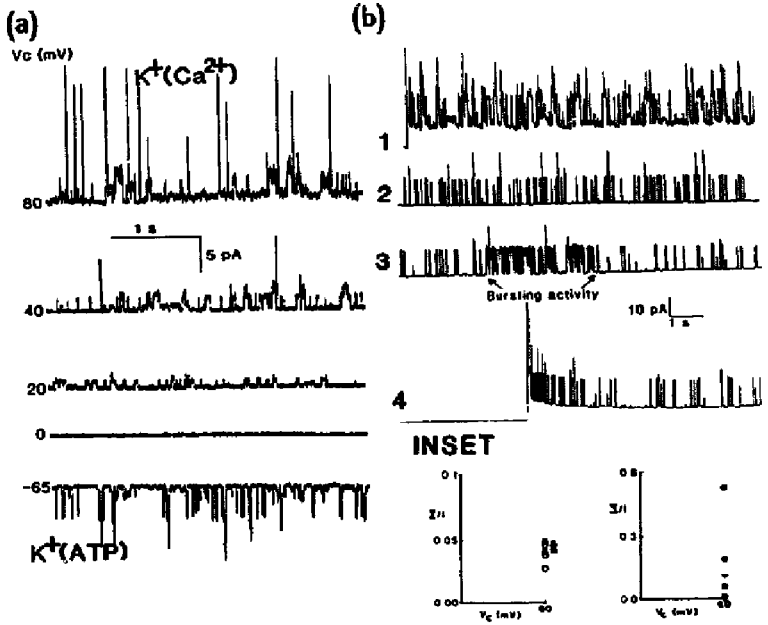

Fig 1 Identification of $K^+(Ca^{2+})$ channels, (a) Current traces displaying typical single K^+ (ATP) and $K^+(Ca^{2+})$ channel currents seen in cell-attached patches of B-cell membrane (Cell-attached patch IS in bath and pipette) (b) Typical time-independent behavior of $K^+(Ca^{2+})$ channel depicting little or no inactivation with sustained depolarization (Traces 1 and 2) Aberrant behavior patterns of $K^+(Ca^{2+}$ channels, namely intermittent 'bursting' activity and time-dependent inactivation after depolarization, are depicted in Traces 3 and 4, respectively (IS pipette, ES bath, traces recorded at $V_c = +60$ mV immediately after (Traces 1,4) or beginning 1 s after (Traces 2 and 3) transition from -90 mV) Insets at the bottom of the figure depict channel activity averaged over 4 s (circles) and 8 s (triangles) for sample patches displaying steady-state (left) and bursting (right) activity patterns Note that, in the absence of obvious bursting, channel activity averaged over short intervals provides a reasonable estimate of average channel activity over longer recordings

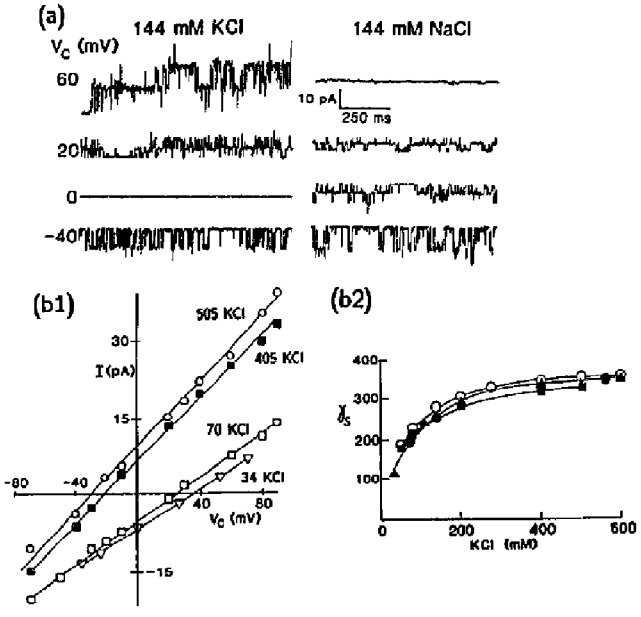

Fig 2. K^+ selectivity of K^+ (Ca^{2+}) channel in the inside-out patches (a) Current traces at various V_c values depicting the shift in single-channel reversal (or zero current) potential from 0 mV to > 60 mV when the bath solution was changed from 144 mM KCl to NaCl in the presence of 0 1 mM Ca^{2+} (IS pipette) (b1) Current–voltage curves displayed by membrane patch after the concentration of KCl of bath was increased from 34 mM (▽) to 70 mM (□) to 405 mM (■) and 505 mM (○) in the presence of 20 mM Hepes-KOH (pH 7 2) (b2) Single-channel conductance γ_s (computed as $\Delta I/\Delta V$ at V_c values near E_{rev} in each solution) presented as a function of the concentration of KCl in three separate experiments

tance channel opening with increasing frequency with increasingly negative V_c values In this condition, altering the bath Ca^{2+} had no effect on channel activity

To standardize nomenclature, the membrane (or clamping) potential, V_c, was defined as the potential at the inner surface of the membrane with respect to ground Analysis of channel amplitude and activity was done using digitized data and an interactive graphics display I/t, or the average number of channels open during a 20–120 s segment of record, was measured from raw data using interactively specified half-amplitude level crossings to determine the fraction of time that 0, 1, 2, 3 or more channels were open

The standard IS used for the pipette and bath consisted of 144 mM KCl/0 1-0 5 mM $MgCl_2$/(no added $CaCl_2$)/Hepes-KOH (pH 7 30) for a final pH of 7 20–7 25 The distilled, deionized water used contained 10–20 μM free Ca^{2+} by Ca^{2+} electrode measurement Substitution of other univalent cations or divalent cations was made by iso-osmotic replacement of KCl In most experiments where channel selectivity was investigated, $MgCl_2$ was removed from the IS and 50–100 μM $CaCl_2$ was added to ensure channel activity over a wide range of membrane voltages The Ca^{2+}-sensitivity of channel activity was calibrated using IS solutions containing various concentrations of $CaCl_2$ and EGTA Free Ca^{2+} concentrations in these solutions were calculated using a computerized nomogram first presented by Fabiato and Fabiato and modified by B.A. Wolf who kindly provided it for our use [16] ES which bathed the cells during initial patching consisted of 138 mM NaCl/5 5 mM KCl/2 mM $CaCl_2$/1 mM Mg^{2+}/Hepes-NaOH (pH 7 35) for a final pH of 7 3

Results

Cation permeation of the Ca^{2+}-activated K^+ channel in excised patches of B-cell membrane

The K^+-selectivity of the large-conductance voltage and Ca^{2+}-activated channel in B cells was demonstrated in two ways First, using inside-out patches symmetrically bathed in IS, we replaced the IS solution in contact with the 'cytoplasmic' surface of the membrane with ES The zero current or reversal potential (E_{rev}) of the 200 pS channel was raised from $V_c = 0$ mV to V_c of at least +60 mV, suggesting that the channel is highly selective for K^+ over Na^+ (Fig. 2), Second, in individual inside-out patches formed with IS-filled pipettes, we

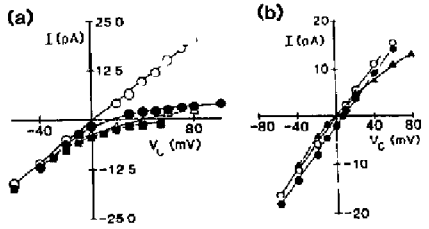

Fig 3 Determination of 'permeability' selectivity sequence of K^+ (Ca^{2+}) channel in the inside-out excised patch (a) Current–voltage curves tabulated for a patch formed with IS in pipette and bath (○) or after iso-osmotic substitution of KCl content of IS bath with RbCl (●), NH_4Cl (△) or LiCl (■) (b) Current–voltage curves tabulated for a similar patch where KCl content of IS bath was iso-osmotically replaced by K^+ acetate (●) or Tl^+ acetate (▲)

systematically altered $[KCl]_i$, the KCl concentration of the solution bathing the cytoplasmic surface, by adding concentrated KCl or replacing a portion of IS with distilled water or sucrose Increasing $[KCl]_i$ from 34 to 506 mM shifted E_{rev} of the current–voltage curves by −67 mV (i e, from +37 mV to −30 mV), this is very close to the 70 mV change in E_{rev} predicted from the Nernst equation for a channel permeable to K^+, but not Cl^- (see Fig 2b1) The single-channel conductance, as well as reversal potential, is highly sensitive to $[K^+]_i$ The slope of the current voltage curves measured at $V_c = 0$ mV increased with $[KCl]_i$ and then saturated at a value near 325–350 pS at $[KCl]_i > 400$ mM (see Fig 2b2). The saturation of channel conductance as a function of $[KCl]_i$ suggests specific interaction of K^+ ions with at least one site in the channel during the process of traversal

To better understand the ability of Ca^{2+}-activated K^+ channels to select among univalent cations, we examined in more detail (a) the 'permeability' selectivity sequence of the channel, and (b) the effects of other ions on K^+ conduction. In four experiments, similar to that shown in Fig. 3a, an inside-out excised patch was formed with IS in the pipette and bath The KCl content of the cytoplasmic bath was then sequentially substituted mole for mole with NaCl, RbCl, LiCl, and NH_4Cl Current–voltage curves were measured in the presence of each cation In two other experiments, represented by the sample in Fig 3b, the selectivity of the channel to thallium was studied by replacing 144 mM potassium acetate with thallium acetate, the acetate salt was used due to the low solubility of TlCl in water E_{rev} was measured as +17 mV in RbCl, +40 mV in NH_4Cl, E_{rev} in thallium acetate was 7 mV negative to that in K^+ acetate Reversal potentials were not measurable in NaCl, LiCl or CsCl (data not shown), as the inward current was not seen to reverse at large depolarizing V_c values In the latter solutions, the lower limit of E_{rev} was estimated as +70 mV by linear extrapolation of the nearest portion of the current/voltage curve obtainable Assuming perfect cation-selectivity, the cationic permeabilities relative to K^+ were calculated under these nearly 'bi-ionic' conditions, using Eqn 1

$$\Delta E_{rev} = \frac{RT}{F} \frac{P_A\ [K^+\ \text{pipette}]}{P_K \cdot [K^+\ \text{bath}] + P_C\ [C^+\ \text{bath}]} \quad (1,$$

where $RT/F = 25.6$ mV and $[K^+\ \text{bath}]$ was the concentration of K^+ (6 mM) in the final K^+ concentration contributed by the Hepes-KOH buffer Thus calculation yielded the following ratios $P_{Tl^+}/P_{K^+} = 1.3$, $P_{Rb^+}/P_{K^+} = 0.5$, $P_{NH_4}/P_K = 0.17$, and P_{Li^+}, P_{Na^+}, $P_{Cs^+}/P_{K^+} \leq 0.05$ These data suggest that, despite its large conductance in the presence of K^+, the channel is highly selective among cations The current–voltage relationship in Tl^+ also demonstrates that though Tl^+ is more 'permeable than K^+, judging from the channel reversal potential, the channel conducts' Tl^+ significantly less well than it 'conducts' K^+ (i e, the maximum slope of the current/voltage curve for outward current in the presence of thallium acetate in the cytoplasmic solution is approx 100 pS, as compared with 200 pS for outward K^+ current) Hence, the relative cation permeability, as calculated from reversal-potential measurements, may be a poor estimator of relative ionic conduction through the channel

Another set of experiments was performed to test how the presence of combinations of cations in the cytoplasmic solution affects channel conduction Fig. 4 demonstrates that addition of either a permeant or impermeant univalent cation (Rb^+ vs Li^+ or Cs^+) to the cytoplasmic solution reduced outward current flow through the channel The reduction in current flow was, in some cases (e g, Cs^+) clearly voltage-dependent, with larger fractional reductions seen with increasing membrane depolarization (or driving force for outward cation movement across the channel) Note that Rb^+,

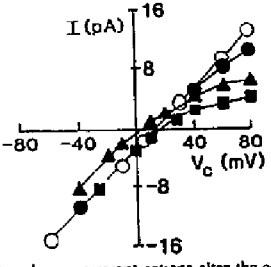

Fig 4 Permeant and non-permeant cations alter the conductance o K^+ (Ca^{2+}) channel in the inside-out excised patch Effects of addition of 80 mM NaCl (●), RbCl (▲) or CsCl (■) to a bath containin; 70 mM KCl 20 mM Hepes-KOH (pH 7 2) and no added Ca^{2+} Not the prominent voltage-dependent reduction in outward current cause by both RbCl and CsCl

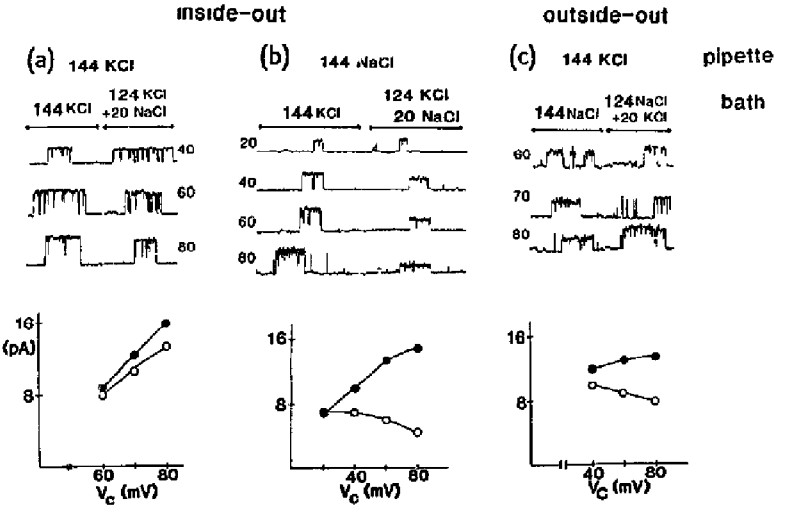

Fig 5 Effects of Na_o^+ and Na_i^+ on channel conductance (a) In an inside out patch formed with 144 mM KCl pipette, replacement of 20 mM KCl in bath with NaCl results in a small voltage-dependent reduction in outward current (b) In a similar patch formed with 144 mM NaCl in pipette, replacement of 20 mM KCl with NaCl results in a profound reduction of outward current and induction of a region of 'negative-slope' conductance (c) In an outside-out patch formed with 144 mM KCl in the pipette and 144 mM NaCl in the bath, iso-osmotically substituting 20 mM KCl for NaCl increases outward current flow through the channel

which is calculated to be at least 7-times more permeant than Na^+, reduces outward unitary current more effectively These results are contrary to the idea of independent movement of ions through the channel, in which case contributions of various ions to current flow should be additive The voltage-dependence of conduction block also suggests that ion 'interaction' is taking place within an electric field (i e , within the membrane)

In physiological conditions, Na^+ is the major impermeant univalent cation available to compete with K^+ for entry into the channel at both the cytoplasmic and external faces of the channel Fig 5 shows the effectiveness of 'cytoplasmic' Na^+, at a concentration approaching physiological intracellular level, in reducing the amplitude of outward current through the $K^+(Ca^{2+})$ channel, as well as the effectiveness of external K^+ in reversing that block In inside-out patches formed with IS in the pipette, substitution of 20 mM KCl of the cytoplasmic IS with NaCl results in a small, voltage-dependent reduction in the amplitude of outward current (Fig 5a) In inside-out patches formed with ES in the pipette, similar substitution of KCl, with NaCl results in more dramatic reductions in single-channel amplitudes at comparable V_c, channel amplitude actually falls

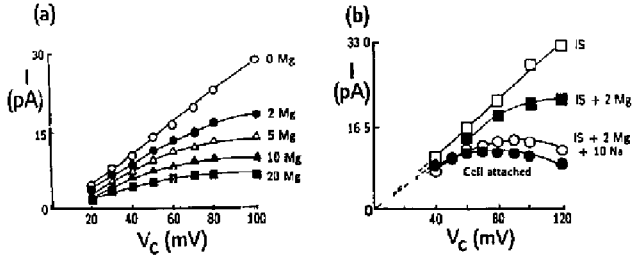

Fig 6 (a) Ability of increasing concentrations of internal Mg^{2+} to progressively block outward conduction through a $K^+(Ca^{2+})$ channel in an inside-out patch. (IS pipette, $MgCl_2$ iso-osmotically substituted for KCl in bath) (b) Addition of small concentrations of Mg^{2+} and Na^+ to the cytoplasmic bath of an inside out excised patch induces inward-going rectification and a region of negative slope conductance resembling that seen in cell attached patch (IS pipette, IS bath (□), IS bath with 2 mM $MgCl_2$ (■), IS bath with 2 mM $MgCl_2$ and 10 mM NaCl (○), cell-attached patch with IS bath (●)

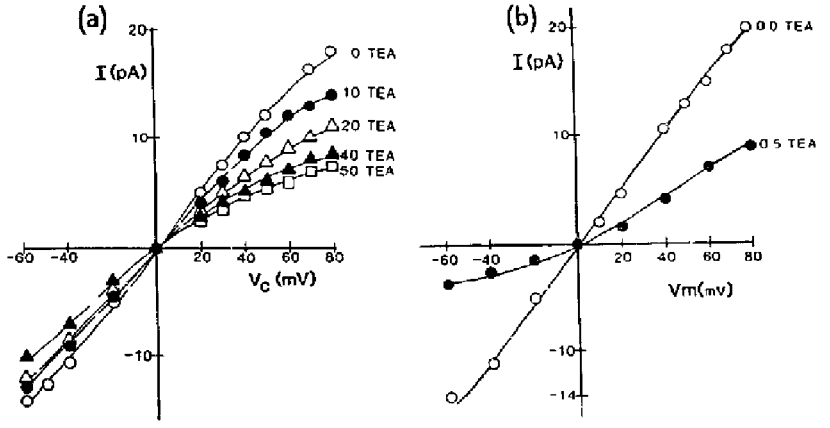

Fig 7 Channel blockade by addition of TEA from internal (left) and external (right) surfaces of K^+ (Ca^{2+}) IS bath and pipette Ins de out patch on left, outside out patch on right V_m is the potential in the pipette with respect to bath ground

with increasing depolarization, hence, producting a region of negative-slope conductance in the current–voltage curve (Fig. 5b) However, in outside-out excised patches formed with ES in the bath and IS in the pipette substitution of 20 mM $NaCl_0$ with KCl is sufficient to increase channel amplitude and remove the negative-slope conductance (Fig 5c) These results suggest that internal Na_i^+ inhibits, while external K^+

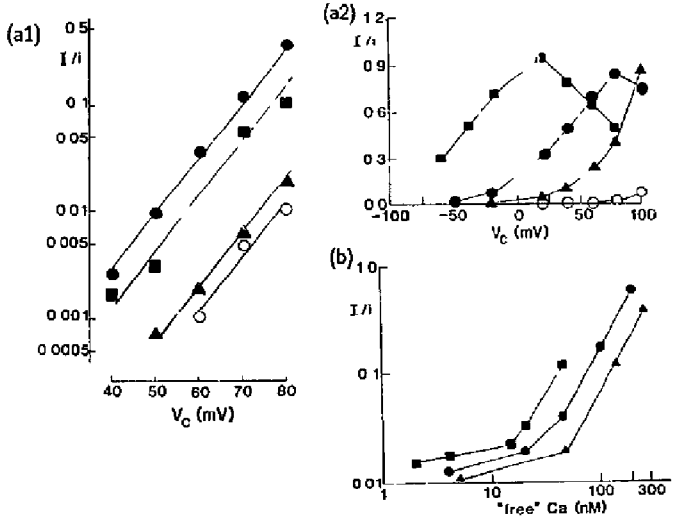

Fig 8 Ca^{2+}-dependent of K^+ (Ca^{2+}) activity in an inside-out excised patch (standard IS pipette) (a) Sample effects of Ca^{2+} on channel activity measured as a function of clamping voltage at low (left) and high (right) calcium concentrations Estimated free Ca^{2+} in parentheses. (a1) ○, 1 mM EGTA IS with no added Ca^{2+} (approx. 5 nM), ▲, +250 μM Ca^{2+} (approx 46 nM), ■, +500 μM Ca^{2+} (approx 140 nM), ●, +650 μM Ca^{2+} (approx 260 nM) (a2) ■ 100 μM Ca^{2+} IS, ●, +80 μM EGTA (approx 22 μM Ca^{2+}) ▲ +100 μM EGTA (approx. 3 5 μM Ca^{2+}) ○, +2 mM EGTA (approx 5 nM Ca^{2+}) (b) Dependence of activity on calculated free Ca concentration in various solutions ▲, Ca^{2+} added in IS containing 1 mM EGTA, ●, Ca^{2+} added in IS containing 1 5 mM EGTA and 0 5 mM ATP and ■, Ca^{2+} added in IS containing 2 mM EGTA and 1 mM ATP

facilitates, outward K^+ flux through this channel These results suggest cooperative interaction of K^+ in channel conduction

Effects of K^+ channel blockers (Mg^{2+} and TEA^+) on conduction through $K^+(Ca^{2+})$ channels

Attention has recently been focused on intracellular Mg^{2+} as a contribution to inward-going rectification displayed to varying degrees by many K^+ channels (see Ref 17) Fig 6a demonstrates that addition of increasing concentration of Mg^{2+} (2–20 mM) to the IS bath of an inside-out patch produces a block of outward current which increases with increasing depolarization (i e, increasing driving force for outward current flow through the channel) Fig 6b demonstrates that, on addition to the IS bath of 2 mM Mg^{2+} and 15 mM Na^+ (i e, concentrations resembling physiological intracellular levels), $K^+(Ca^{2+})$ channels display inward rectification similar to that seen in cell-attached patches

Alkyl-substituted ammonium ions, such as tetraethylammonium, are often-used K^+-channel blockers Their blocking action is often voltage-dependent and more potent when applied to one side of the channel or the other Fig 7 compares the action of TEA on $K^+(Ca^{2+})$ channel currents after its application to the 'cytoplasmic' surface of an inside-out patch (a) and its application to the external surface of an outside-out patch (b) External TEA is roughly 100-fold more potent in reducing inward current than internal TEA is in reducing outward current (Compare the K_d of 0 5 mM for TEA_o with 10 mM for TEA_i)

These results suggest that the pathway of approach of ions from the cytoplasmic and external membrane surfaces to the saturable site(s) within the channel, which regulates ion flow, may be asymmetric

Gating by divalent cations of the Ca^{2+}-activated K^+ channel in excised patches of C-cell membrane

In survey experiments [13], we have demonstrated that the large-conductance, voltage-dependent K^+ channel of the B cell was sensitive to cytoplasmic Ca^{2+} over the nanomolar to micromolar range In several well-controlled experiments, we attempted to quantitate this Ca^{2+}-sensitivity As demonstrated by the sample experiments and compilation graph of Fig. 8, the $K^+(Ca^{2+})$ channel is indeed sensitive to $[Ca^{2+}]_i$ over a concentration range spanning several orders of magnitude Several prominent features emerge from these experiments (1) The Ca^{2+}-sensitivity of the channel is low at $[Ca^{2+}]$ values below 20–50 nM, but increases substantially at $[Ca^{2+}]$ values between 50 nM and 1 μM, where I/i is proportional to $[Ca^{2+}_{free}]^{1-2}$

Within most of this range of calcium concentrations, channel activity is voltage-dependent, with mean channel activity maximally increasing e-fold per 10–12 mV incremental depolarization (2) At $[Ca^{2+}]$ values greater than 10 μM, channel activity shows reduced voltage and Ca^{2+}-sensitivity, channel activity sometimes even decreases with increasing depolarization Examination of the latter current records reveals the development of intermittent long pauses in channel activity (3) $[Ca^{2+}]_i$ values greater than 10–20 μM were needed to produce even detectable channel activity at V_m equal to or negative to 0 mV The Ca^{2+}-sensitivity seen here resembles that previously found with $K^+(Ca^{2+})$ channels in neonatal rat islet [10] Apparently, higher Ca^{2+}-sensitivities have been previously reported for adult rat islet based on solutions where EGTA Ca concentration ratios were near 1 . 1 [11]

To better understand the nature of Ca^{2+} gating, we have investigated whether other divalent cations can substitute for Ca^{2+} Rather than chelating Ca^{2+} and then adding other divalent cations, we added test concentrations of a variety of divalent cations to the small but fixed concentration of Ca^{2+} (≈ 15 μM) contained in no added Ca^{2+} IS In Fig 9, which is representative of

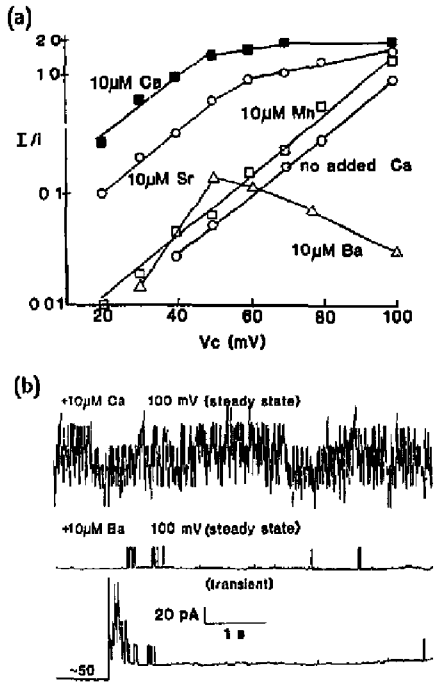

Fig 9 (a) Effects of internal application of various divalent cations on voltage dependent gating of $K^+(Ca^{2+})$ channel (IS pipette and IS bath with added chloride salts of divalents as indicated) (b) Ability of 10 μM Ba^{2+} to produce a time-dependent block of activity of $K^+(Ca^{2+})$ channel at large depolarizing voltage, hence producing a pattern of activity resembling voltage-dependent channel inactivation Steady-state effect of 10 μM Ca^{2+} shown for comparison

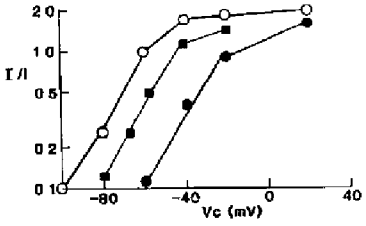

Fig 10 Internal application of NBA reduces $[Ca^{2+}]_i$-sensitivity of $K^+(Ca^{2+})$ channel in the inside out excited patch without altering its voltage dependence (IS pipette IS+100 μM Ca^{2+} bath) Control (○), +100 μM NBA (●), +100 μM NBA+1 mM $CaCl_2$ (■)

three experiments, addition of 10 μM Sr^{2+} increased mean channel activity more than 10-fold above that in no-added Ca^{2+}, but only 30% as much as did the addition of 10 μM Ca^{2+} In contrast, addition of 10 μM Mn^{2+} only increased mean channel activity 2-fold above the no-added Ca^{2+} condition Note that the voltage-dependence of channel activity was not altered The effect of Ba^{2+} is considerably more complicated Addition of Ba^{2+} significantly increases channel activity at negative V_c, but decreases activity at positive V_c values Fig 9b suggests that one origin of Ba^{2+} inhibition of channel activity may be due to time and voltage-dependent channel block by Ba^{2+} The block appears to be dependent on the magnitude of the electrochemical gradient driving Ba^{2+} into the channel Hyperpolarization transiently relieved the steady-state block

Experiments of the type depicted in Figs 8 and 9 demonstrated a rather characteristic voltage-dependence of channel gating over a wide range of divalent cation concentrations, suggesting that, at least under some conditions, voltage and ion gating of the channel might be independent (Under other conditions, however, such as the presence of cytoplasmic Ba^{2+} or $[Ca^{2+}]_i$ greater than 20 μM, voltage and divalent cation gating were related through voltage-dependent block of the channel by gating cations.) Recently, Pallotta [18] has reported that small concentrations of *N*-bromoacetamide (NBA) made the $K^+(Ca^{2+})$ channel of myotubes nearly insensitive to Ca_i^{2+} Hence, in another set of experiments, we added 100 μM NBA to the cytoplasmic bathing solution of an inside-out patch, which also contained either 10 or 100 μM $CaCl_2$ In 10 μM $CaCl_2$, we noted a progressive reduction in channel activity with time, but after 10 min, single-channel currents became rather 'ragged' In three such experiments using 100 μM $CaCl_2$ (see, for example, Fig 10), we were able to examine the voltage-dependence of channel activity in fixed Ca_i^{2+} before and 10 min after addition of 100 μM NBA Fig. 10 shows that, while the Ca^{2+}-sensitivity is clearly reduced, the characteristic voltage-dependence of channel activity and the maximum average number of channels activated is basically unchanged This further suggests that Ca^{2+} and voltage activation of the channel can be independently altered under certain circumstances

Effect of pH_i on activity of $K^+(Ca^{2+})$ channel

In previous experiments, $K^+(Ca^{2+})$ channels in membrane patches excised inside-out from rat neonatal islet cells displayed increased activity with alkalinization of the 'cytoplasmic solution' (from pH 7 2 to 7 8) and decreased activity with acidification (from pH 7 2 to 6 8) [10] However, in our recordings from cell-attached patches, addition of NH_4Cl to a KCl bath, which should result in prolonged intracellular alkalinization, produced no discernable effect on $K^+(Ca^{2+})$ channel activity [19]

A possible reconciliation of this discrepancy is presented in Fig 11 In part (a) $K^+(Ca^{2+})$ channel activity

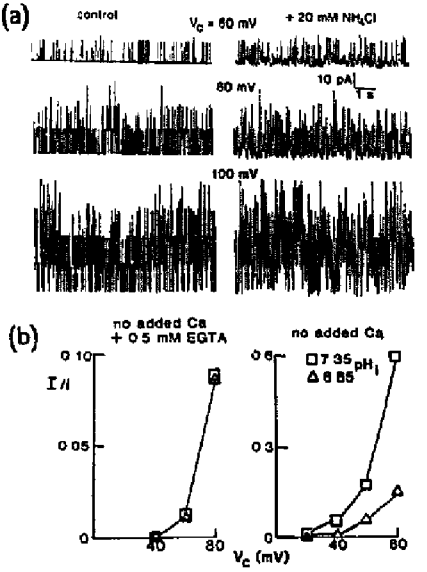

Fig 11 (a) Lack of effect of cell alkalinization, produced by bath addition of 20 mM NH_4Cl, on $K^+(Ca^{2+})$ channel activity in the cell-attached patch (symmetric IS in the pipette and bath) (b) Variable effect of pH_i on gating of $K^+(Ca^{2+})$ channel in inside-out excised patch. (IS pipette modified IS bath containing 0 5 mM $MgCl_2$ and 0 5 mM ATP) No reduction in channel activity was seen when pH_i was reduced from 7 35 to 6 85 in IS containing no added Ca and 0 5 mM EGTA (left) but a nearly 4-fold reduction in channel activity was seen with an identical reduction in pH_i in the absence of EGTA (right) In the presence of EGTA, reducing pH_i is calculated to increase free Ca^{2+} roughly 7–8 fold from approx 6 to 46 nM Judging from Fig 8b, this should increase I/i by 2–7-fold in the absence of any counteracting effect. This is, however, roughly comparable to the decrease in I/i seen with the same maneuver in the absence of the EGTA buffer

in the cell-attached patch appears to be unaffected by addition of 20 mM NH_4Cl, though the activity of the K^+(ATP) channel is greatly enhanced, as indicated by the increase in activity of smaller conductance channels Following excision of the patch in the inside-out configuration (Fig 11b), reducing pH_i from 7 35 to 6 85 reduced $K^+(Ca^{2+})$ channel activity in the absence, but not the presence, of EGTA The buffering capacity of EGTA is strongly pH-dependent Hence, it is possible that, in the presence of EGTA, the increased effectiveness of Ca_i^{2+} in gating the channel, otherwise seen at higher pH_i, is largely countermanded by the reduction in free Ca^{2+} resulting from enhanced chelation, when H^+ is less available to compete at a Ca^{2+} chelation site Cytoplasmic buffers might function in a similar manner

Discussion

We have described a variety of features of ionic selectivity and gating of a large-conductance Ca^{2+}- and voltage-activated K^+ channel in patches of plasma membrane excised from pancreatic islet B cells (1) These channels have an overwhelming selectivity for K^+ over Cl^- (2) Channel conductance in inside-out excised patches formed with isotonic KCl pipettes increases with bath K^+, but appears to saturate simply at approx 350 pS at KCl concentrations higher than 400 mM (3) The permeability selectivity sequence ratio to K^+ as determined from the Goldman-Hodgkin-Katz equation was 1.3 Tl^+ 1 0 K^+ 0 5 Rb^+ 0 17 nH_4^+ < 0 05 Na^+, Li^+ or Cs^+ (4) Permeant alkali earth cations (e g., Tl^+ and Rb^+) reduce conduction through the channel Impermeant cations are also able to do this For example, Na^+ and Mg^{2+} when added to the cytoplasmic side of the membrane reduce the outward flux of ions, hence, offering a clue to the origin of inward-going rectification displayed by the channel in cell-attached patches The well-established K^+-channel blocker, TEA, was also tested, it was found to be a more potent channel blocker from the external side ($K_d \leq 0$ 5 mM) than the cytoplasmic side ($K_d \approx 30$ mM) (5) The channel is sensitive to $[Ca^{2+}]_i$ over a wide range (< 5 nM to > 100 μM), over the range 50–500 nM, which is the range over which average, free cytosolic Ca^{2+} varies for most cells, channel activity varies as the 1 5–1 6 power of $[Ca^{2+}]_i$. (6) In the presence of Ca^{2+}, addition of divalent cations enhances channel activity with an order of potency $Ca^{2+} > Sr^{2+} > Ba^{2+} > Mn^{2+}$, Co^{2+} The effect of Ba^{2+} is complicated by its ability to produce time-dependent channel inactivation at potentials at which it is driven into the membrane (7) Channel activity shows a characteristic voltage-dependence, maximally increasing e-fold per each 10–12 mV incremental depolarization, this characteristic persists over a wide range of Ca^{2+} concentrations, and even during augmented activation by other divalent cations (8) The channel is sensitive to pH_i, at fixed $[Ca^{2+}]_i$, cytoplasmic alkalinization increases activity Features (1), (2), (3), (4), and (7) closely resemble those reported in detail for the large-conductance $K^+(Ca^{2+})$ channel in inside-out excised patches from cultured rat muscle sarcolemma [4,20] The voltage and Ca^{2+}-dependence of channel gating at low levels of channel activity most closely resemble those of the similar conductance $K^+(Ca^{2+})$ channel in cultured hippocampal neurons [21] The channel described here shares features also displayed by 'maxi' $K^+(Ca^{2+})$ channels seen in many other cells, such as adrenal chromaffin cells [3,21,22], renal cortical collecting tubule cells [24] and amphibian gastric smooth muscle cells [25] In the latter cell types, however, 'maxi' K^+ channels in the cell-attached patch are often open over a wider range of V_c values than what is seen with the channels in the B cell

The 'maxi' $K^+(Ca^{2+})$ channel in native and reconstituted membrane patches has been a favorite channel for permeation studies, because its currents are easily recorded over a wide range of conditions and because its very large conductance poses the paradox of how selectivity can be maintained as ions traverse the channel at a surprisingly fast rate For example, if the channel were 60 Å in length by 6 Å in diameter (or large enough to span the membrane and accommodate a hydrated K^+ ion), its calculated conductance in symmetric 120 mM KCl solution would be approx 50 pS, the actual conductance is more than 4-fold higher (see Hille, Ref 2 and Yellen, Ref 26) While there is no direct knowledge of the conductance mechanisms of a real $K^+(Ca^{2+})$ channel, experiments such as those described here suggest that ion traversal is far more complex than free ionic diffusion through a fluid-filled pore (1) The concentration vs conductance curve of the channel (Fig 2b) suggests at least one 'saturable' selectivity site within the channel which permits passage of K^+, Tl^+ and Rb^+ and, only grudgingly, NH_4^+ (2) The voltage-dependent reductions in channel current by impermeant and permeant cations alike suggest that K^+ and other ions interact at a site which experiences an electric field (i e , within the membrane) and that ions cannot pass each other at that site (3) The ability of large-diameter ions (i.e., alkyl-substituted NH_4^+ ions) to reduce conduction with different potencies from the outside or the inside surface of the membrane suggests that pathways for approach to the intramembrane binding site may be vestibule or antechamber-like and also asymmetric (4) The ability of small concentrations of external K^+ to partially relieve block of K^+ efflux caused by internal Na^+ (i e , a 'trans-K^+' effect) (see Fig 5) is consistent with the long-distance interaction between K^+ ions in the channel, perhaps by mutual electrostatic repulsion between ions waiting or binding at the saturable sites [26]

Others have modelled similar data for the 'maxi' $K^+(Ca^{2+})$, either in terms of a single-ion channel with

two Eyring rate barriers and an intervening low-energy well containing an ion binding site [20], or as a multi-ion channel with two low-energy wells, each of which can simultaneously accommodate one ion [23,26,27] From our date, the strongest evidence in favor of a two-ion channel is the 'trans-K^+' effect Two other pieces of our evidence, which might ne used to distinguish a 'two-ion' from a 'one-ion' channel model are apparently more consistent with a one-ion channel model (1) The channel conductance vs K^+ concentration curve appears to saturate as a simple hyperbola, at least over the range examined (2) At constant total $Rb^+ + K^+$, varying the Rb^+/K^+ ratio (or 'mole fraction') does not alter the P_{Rb^+}/P_{K^+} calculated from the E_{rev} seen at each combination (see Tabcharani et al , Ref 13, data not presented here) (It has been argued that Cs^+ may be a better ion for the examination of possible anomalous mole-fraction features [27] In our experiments, Cs^+ increased channel noise and patch instability)

The joint gating of the 'maxi' $K^+(Ca^{2+})$ channel by transmembrane voltage and cytoplasmic Ca^{2+} has intrigued investigators since the discovery of the channel As nanomolar concentrations of Ca_i^{2+} alter channel activity even in the presence of millimolar concentrations of Mg^{2+}, electrostatic screening by Ca^{2+} of the negative surface charge of a voltage-sensitive 'gate' is an unlikely mechanism for Ca^{2+}-sensitivity Is the exquisite Ca^{2+}-sensitivity due to the presence of a Ca^{2+}-binding region of the channel resembling other Ca^{2+}-binding proteins? How is such a region functionally linked to the voltage gate? Our data support the idea that over a wide range of function, voltage and Ca^{2+} gating of the channel may be somewhat independent First, the maximum voltage-dependence of Ca^{2+} activity is not altered over a wide range of $[Ca^{2+}]_i$ (approx 10^{-9}–10^{-5} M) Second, the Ca^{2+}-sensitivity of the channel can be greatly reduced by treatment with *N*-bromoacetamide, a protein modifying agent which cleaves peptide bonds on the COOH terminal side of several amino acids, without affecting voltage-sensitivity Establishment of the sensitivity of the channel to a variety of divalent cations might be used to compare the Ca^{2+} gate with isolated Ca^{2+} binding proteins. Our data suggest that Sr^{2+} and Ba^{2+} can augment the channel gating by Ca^{2+}, but do not distinguish whether these ions actually substitute for Ca^{2+} with varying efficiency, or change the binding affinity for Ca^{2+} In a more thorough study using bilayer membranes reconstituted from $K^+(Ca^{2+})$ channel-containing membrane vesicles from muscle sarcolemma [29], the following order of potency of channel activation by divalent cations was established $Ca^{2+} > Sr^{2+} > Cd^{2+} > Mn^{2+} > Fe^{2+}$, Ba^{2+} was ineffective This rank order is similar for divalent cation binding by calcium-binding proteins, troponin C, calmodium and parvalbumin. In the presence of Ca^{2+}, a variety of divalent cations, including Cd^{2+}, Co^{2+}, Mn^{2+}, Ni^{2+} and Mg^{2+}, increased the apparent affinity of the channel for Ca^{2+} by increasing the Ca^{2+}-dependence of the channel (i e the Hill coefficient) in a concentration-dependent manner A more fruitful long-term approach to the problem of Ca^{2+} binding may involve examination of the homology of the sequence and three-dimensional structure of the $K^+(Ca^{2+})$ channel and the Ca^{2+} binding proteins A first step in this approach would be the isolation of the $K^+(Ca^{2+})$ channel, perhaps as a charybdotoxin-binding protein [30]

The function of the $K^+(Ca^{2+})$ channels in B cells remains uncertain, despite their abundance It is now generally appreciated that ATP-sensitive K^+ channels, rather than the $K^+(Ca^{2+})$ channels (1) underlie the resting potassium permeability P_{K^+} which is regulated by cell metabolism [31], and (2) are the specific targets for pharmacological agents (e g., sulfonamides) and physiological maneuvers (e g changes in intracellular pH) which alter P_K [19 31] The data on the Ca^{2+}-dependence of channel activity presented here suggest that free cytosolic Ca^{2+} would have to increase into the range of at least several micromolar for channel activity to be detectable at membrane potentials approaching normal V_{rest} This is supported by data from companion experiments on channel activity in cell-attached patches where the remainder of the cell membrane has been permeabilized to Ca^{2+} by the ionophore ionomycin in the presence of 0 1–1 mM Ca^{2+} [12], under these conditions, $K^+(Ca^{2+})$ channel activity was not seen at V_m values negative to 0 mV in patches containing four or five such channels, even though average free intracellular $[Ca^{2+}]$ would be expected to rise to a level equal to or greater than one to several micromolar These experiments make it less likely that $K^+(Ca^{2+})$ channels play a straightforward role in secretogogue-induced electrical activity

Acknowledgements

We thank the laboratory of Michael McDaniel (Department of Pathology Washington University) for generously providing cells, Betty Yizama for preparing the typescript, and most of all, Lee Falke and Kevin Gillis for stimulating discussions, camaraderie and efforts at modelling these data which aided our thinking This work was presented by J A T. to the Sever Institute of Technology, Washington University (May, 1987), in partial fulfillment of requirements for a Master of Science degree in Biomedical Engineering. The work was supported by NIH grant R01DK37380 and a Washington University Medical School Pre-Clinical Teacher of the Year Award (1986-1987) to S M

References

1 Meech R W (1974) J [illegible] 23[illegible], 259–277
2 Hille B (1984) Ionic Channels of Excitable Membranes Sinauer, Sunderland, MA

3 Marty A (1981) Nature 291 497–500
4 Barrett, J N, Magleby, K L and Pallota, B S (1982) J Physiol 331 211–230
5 Wong, B S, Lecar, H and Adler, M (1982) Biophys J 39 313–317
6 Blatz, A L and Magleby, K L (1987) Trends Neurosci 10, 463–467
7 Atwater, I, Dawson, C M, Ribalet, B and Rojas, E (1978) J Physiol 278 117–139
8 Findlay, I Dunne M J and Petersen, O H (1985) J Membr Biol 88 165–172
9 Ashcroft, F Ashcroft, S L and Harrison (1988) J Physiol 400, 501–527
10 Cook, D L, Ikeuchi M and Fujimoto W Y (1984) Nature 311 269–271
11 Findlay, I Dunne, M J and Petersen, O H (1985) J Membr Biol 83, 169–175
12 Misler, S, Falke, L, Gillis, K, Hammoud, A and Tabcharani J (1988) J Gen Physiol 92, 7a–8a
13 Tabcharani J, Falke L and Misler, S (1987) Biophys J 51, 52a
14 Misler, S, Tabcharani J and Gillis K (1987) Biophys J 51, 52a
15 Misler S Falke L C, Gillis, K and McDaniel M L (1986) Proc Natl Acad Sci USA 83, 7119–7123
16 Wolf, B A, Turk, J, Sherman, W R and McDaniel, M L (1986) J Biol Chem 261, 3501–3511
17 Stanfield, P R (1988) Trends Neurosci 11, 475–477
18 Pallota, B S (1985) J Gen Physiol 86 601–611
19 Misler, S, Gillis, K and Tabcharani J (1989) J Membr Biol in press
20 Blatz, A L and Magleby, K L (1984) J Gen Physiol 84 1–23
21 Franciolini, F (1988) Biochim Biophys Acta 943, 419–427
22 Yellen, G (1984) J Gen Physiol 84, 157–186
23 Yellen, G (1984) J Gen Physiol 84, 187–199
24 Cornejo, M, Guggino, S E and Guggino, W B (1987) J Membr Biol 99, 147–155
25 Singer, J J and Walsh, J V (1987) Pfluegers Arch 408, 98–111
26 Yellen, G (1987) Annu Rev Biophys Chem 16, 227–246
27 Latorre R (1986) in Ion Channel Reconstitution (Miller, C ed) pp 431–467, Plenum Press, New York
28 Cecchi, X, Alvarez, O and Wolff, D (1986) J Membr Biol 91, 11–18
29 Oberhauser A, Alvarez, O and Latorre, R (1988) J Gen Physiol 92, 67–86
30 Miller, C, Moczydlowski E, Latorre, R and Phillips M (1985) Nature 313 316–318
31 Ashcroft, F (1988) Annu Rev Neurosci 11, 97–118