

Metabolic Regulation of the K(ATP) and a Maxi-K(V) Channel in the Insulin-Secreting RINm5F Cell

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ABSTRACT K channels in the cell membrane of the insulin-secreting RINm5F cell line were studied using the patch-clamp technique in cell-attached patch mode. With 140 mM K in the pipette, two channels displaying different conductive and kinetics properties were observed. A voltage-independent, inward-rectifying, 55-pS channel was active at rest (no glucose, -70 mV), but was almost completely inhibited by 5 mM glucose. A 140-pS channel was seen in the absence of glucose only after cell membrane depolarization with high (30 mM) K. This channel was voltage dependent, with a linear slope conductance between -60 and $+60$ mV, and was completely inhibited only by >15 mM glucose. The former channel we identify as an ATP-sensitive channel previously described in excised patches and refer to it as the K(ATP) channel. The latter, because of its large conductance and voltage-dependent kinetics, will be referred to as the maxi-K(V) channel, adopting a nomenclature previously used to classify highly conductive K channels (Latorre, R., and C. Miller, 1983, *Journal of Membrane Biology*, 71:11-30). In addition to glucose, mannose and 2-ketoisocaproate, which also initiate insulin secretion and electrical activity in the islet β cell, reduced the activity of both the K(ATP) and the maxi-K(V) channel. Lactate and arginine, which potentiate but do not initiate insulin secretion or β cell electrical activity in normal islets, each caused a large reduction in maxi-K(V) channel activity, without consistently affecting the activity of K(ATP) channels. Another agonist that potentiates insulin secretion and electrical activity in normal cells, the tumor-promoting phorbol ester TPA, blocked maxi-K(V) channel activity while stimulating the activity of the K(ATP) channel, thereby implicating phosphorylation in the control of channel activity. These results indicate that metabolic substrates that initiate electrical activity and insulin secretion in normal β cells reduce the activity of both the K(ATP) and the maxi-K(V) channel, while potentiating agents reduce only the maxi-K(V) channel. The possible role of these two channels in the processes of initiation and potentiation of the β cell response is discussed.

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INTRODUCTION

Studies of the pancreatic β cell using glass microelectrodes have described the membrane potential response to glucose in detail (Meissner and Schmelz, 1974; Beigelman et al., 1977). The early phase of this response consists of an initial depolarization of 15–20 mV from a resting potential of around -70 mV elicited by glucose concentrations in the range 4–8 mM (this varies from cell to cell). Higher concentrations of glucose lead to slow membrane potential oscillations between a silent phase potential at around -55 mV and a plateau potential ~ 15 mV more depolarized, from which trains of Ca action potentials arise. As the glucose concentration is increased in the range 8–20 mM, the silent phases between plateaus progressively shorten until they are eliminated and action potentials occur continuously (Beigelman et al., 1977).

The significance of K permeability regulation in stimulus-secretion coupling in the β cell was initially demonstrated in radioisotope studies (Sehlin and Taljedal, 1975). These experiments showed that a decrease in Rb efflux was associated with glucose-induced insulin secretion. Since it was known that the β cell membrane potential depolarizes upon addition of glucose, it was proposed that this depolarization resulted from a reduction of the cell membrane K permeability (Atwater et al., 1978; Meissner et al., 1978). This proposal was strengthened by the observation that the β cell input resistance rises concomitantly with the depolarization (Atwater et al., 1978). Initial hypotheses to explain this observation were based upon the assumption that a Ca-activated K permeability was involved in the depolarization, since the β cell could be depolarized from rest with quinine (then thought to block this permeability specifically) and hyperpolarized in the presence of glucose by application of metabolic uncouplers or inhibitors (the effect of which was proposed to be via the intracellular liberation of stored Ca) (Atwater et al., 1979).

The presence of a K channel that was active in the unstimulated β cell and was blocked by addition of glucose (Ashcroft et al., 1984) and its subsequent identification with the ATP-sensitive K channel (Rorsman and Trube, 1985; Dunne et al., 1986; Mislner et al., 1986; Ashcroft et al., 1987) previously characterized in isolated β cell membrane patches (Cook and Hales, 1984) necessitated a re-evaluation of the role of K permeability in β cell stimulus-secretion coupling. This channel will be referred to as the K(ATP) channel in this article. It is currently believed that the initial depolarization observed upon application of glucose results from blocking of this channel, after an increase in the intracellular ATP level (Rorsman and Trube, 1985; Dunne et al., 1986; Mislner et al., 1986; Ashcroft et al., 1987). Conversely, the hyperpolarizing effect of metabolic uncouplers and inhibitors results from ATP depletion rather than from a rise in intracellular free Ca, since the K(ATP) channel is activated by the mitochondrial uncoupler 2,4-dinitrophenol (DNP) (Ribalet and Ciani, 1987) and is insensitive to Ca (Cook and Hales, 1984). Previous studies from this laboratory, as well as those of Mislner et al. (1986), have shown that the K(ATP) channel is blocked by glucose concentrations as low as 5 mM, which indicates that the role of this channel might be confined to the initial cell depolarization and that it would not influence the burst pattern. In this case, any hyperpolarizing mechanism during the excitable phase of the electrical activity (such as is required to account for both the falling phase of the action potential and the sustained hyper-

polarization between bursts) must be due either to inactivation of excitability mechanisms (e.g., of Ca and Na channels) or to activation of K channels different from the K(ATP). Evidence for the latter alternative is provided by our finding, in cell-attached patches of RINm5F cells, of a K channel that is unambiguously distinct from K(ATP): it has a much larger conductance, the activity depends on voltage, and, in cell-attached patches, it manifests itself consistently only when the cell membrane potential is depolarized using high K (30 mM) in the bath. We shall refer to this channel as a β cell maxi-K(V) channel.

The experiments reported in this article were performed to examine more systematically the effect of glucose on K(ATP) channel activity and to investigate whether glucose exerts a modulatory influence on the maxi-K(V) channel. In addition, the effect of several other modulators of insulin secretion in the normal β cell, both initiators and potentiators, was investigated. In this instance, initiators are compounds that initiate the secretory response at low concentrations and modulate it at higher concentrations, while potentiators can only modulate the response if an initiator is also present (Ashcroft, 1981). The purpose of this study was to determine whether the differentiation of metabolic substrates into these categories was reflected by their different abilities to affect the activity of the two channels. In addition, the tumor-promoting phorbol ester 12-*O*-tetradecanoly-phorbol-13-acetate (TPA), which functions as a potentiator in normal cells, was used to examine whether protein phosphorylation was involved in K channel modulation.

MATERIALS AND METHODS

Cell Culture

Cells of the insulin-secreting cell line RINm5F (kindly supplied by A. E. Boyd III, Baylor College of Medicine, Houston, TX) were maintained at 37°C in RPMI 1640 medium supplemented with 10% (vol/vol) heat-inactivated fetal calf serum, 100 U/ml penicillin, 100 μ g/ml streptomycin, and 2 mM glutamine. The cells were divided once a week after treatment with trypsin and plated onto glass coverslips in 4-cm-diam petri dishes for transfer to the experimental chamber. Cells were used 3–7 d after plating onto glass. 1 h before the beginning of the experiment, a petri dish of cells was removed from the incubator and the solution bathing the cells was replaced with glucose-free bath solution (described below); thereafter, the cells were maintained at room temperature.

Experimental Media

For preliminary incubation and for studies of the K(ATP) channel, the composition of the bath solution was as follows: 135 mM NaCl, 5 mM KCl, 2.5 mM CaCl₂, 1.1 mM MgCl₂, 10 mM HEPES, with the pH adjusted to 7.2 with 1 M NaOH. For studies of the maxi-K(V) channel, KCl was increased to 30 mM with a compensating decrease in NaCl. Glucose and other agents were added directly to the bath solution, except for TPA, which was added from a stock solution in dimethylsulfoxide (DMSO). The final concentration of DMSO in the bath was 0.015%, a concentration that had no effect on the activity of either channel. The pipette contained 140 mM KCl, 1.1 mM MgCl₂, 2.5 mM CaCl₂, and 10 mM HEPES, with the pH adjusted to 7.2 with 1 M KOH.

Data Handling

The production of patch electrodes and the circuitry for recording from them have been described in detail (Hamill et al., 1981). Single-channel currents were recorded at 2 kHz with either an EPC-7 (List Electronic, Darmstadt, Federal Republic of Germany) or an Axopatch 1A (Axon Instruments, Inc., Burlingame, CA) patch-clamp amplifier, and data were stored digitally via digital audio processor and VCR. For analysis, data were acquired to the fixed disk of IBM PC/AT computers via double-memory buffer interface, which permits transfer of data points in a continuous mode (Bezanilla, 1985). Data were acquired to the fixed disk at either 5 or 10 kHz. Amplitude histograms of channel current were constructed to measure the mean channel current. The percentage of channel activity was determined after computation of an idealized data record, using the half-amplitude threshold method (Colquhoun and Sigworth, 1983). Since the control activity of the individual channels may vary widely in terms of absolute percentage of activity, the data were normalized such that the control level of activity was 100%, in order to permit comparison between individual experiments.

RESULTS

Identification and Glucose Sensitivity of the K(ATP) Channel

In cell-attached patch experiments using RINm5F cells, a single type of K-selective channel was routinely observed in the absence of glucose. This channel was identified as the glucose-sensitive K channel seen in intact rat pancreatic β cells and the ATP-sensitive channel observed in isolated patches from rat β cells (Petersen and Findlay, 1987). On the basis of the great similarity of their conductive and kinetic properties, it was proposed that these represent the same channel, referred to here as the K(ATP) channel. The following studies were carried out to further characterize the K(ATP) channel in RINm5F cells.

Conductance and kinetic properties of the K(ATP) channel. Fig. 1 A illustrates the current-voltage (*I-V*) properties of the K(ATP) channel activity shown in Fig. 1 B. This activity was recorded in the absence of glucose. Patch potential is defined as the difference between membrane potential (V_m) and pipette potential (V_p). From the zero-current potential, the cell resting potential was estimated to be -75 mV, assuming that intracellular K is 140 mM. From eight experiments, the mean and standard deviation for the reversal potential were calculated as -70.7 ± 4.10 mV. This is close to the resting potential and also the K equilibrium potential measured in β cells in normal mouse islets of Langerhans using intracellular microelectrodes (Meissner and Schmelz, 1974; Beigelman et al., 1977). The accuracy of this estimate is reinforced by the observation that addition of the metabolic uncoupler dinitrophenol (DNP) (10^{-5} mM), which rapidly reduces cellular ATP levels and thereby enhances the activity of the K(ATP) channel, had no effect on the zero-current potential measured from the *I-V* plot (Ribalet and Ciani, 1987).

The *I-V* relationship was linear for the inward current with a slope conductance of 49 pS, while it saturated for the outward current. A mean value of 55.1 ± 5.2 pS was deduced from eight experiments. This is in close agreement with published values for the K(ATP) channel conductance in the rat β cell (Misler et al., 1986) and in RINm5F cells for the larger of the two glucose-dependent K channels (Dunne et al., 1986).

The inset to Fig. 1 A is a plot of percentage channel activity vs. patch potential for

the data shown in Fig. 1 *B*. These data demonstrate the absence of any significant voltage dependence, with the percentage of channel activity varying in a nonsystematic manner between 11 and 18%, and they are in agreement with previous observations of the absence of voltage dependence of the K(ATP) channel (for review, see Petersen and Findlay, 1987).

The dynamic response of the K(ATP) channel to glucose. Stimulation of RINm5F cells with glucose causes a decrease in K(ATP) channel activity. This effect is illustrated in the lower part of Fig. 2, where the addition of 2.5 mM glucose led to a rapid reduction in channel activity to ~10% of its control value. Consistent results have been described previously in normal rat pancreatic β cells by Ashcroft et al. (1984) and by Rorsman and Trube (1985). However, the latter authors reported the

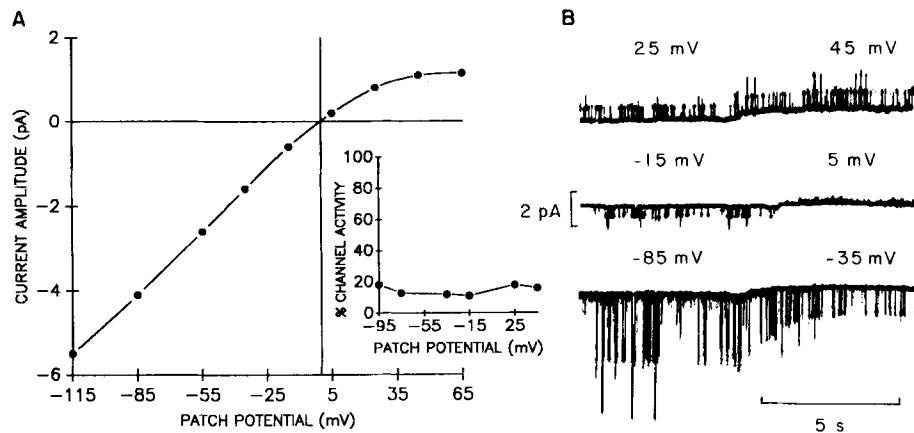


FIGURE 1. The effect of patch potential on the K(ATP) channel. (A) Single-channel current as a function of voltage in a cell-attached patch with 140 mM K in the pipette and 5 mM K in the bath. The magnitude of the membrane potential (V_m) was estimated from the pipette potential (V_p) at zero current, assuming an intracellular K concentration of 140 mM. In this case, V_m was -75 mV. The patch potential was calculated as $V_m - V_p$. The slope conductance in the linear region of the plot was 49 pS. The inset shows the percentage of channel activity in the same patch as a function of patch potential. (B) Representative examples of single-channel current recordings from the same experiment as illustrated in A. The downward deflections of the trace indicate inward current. The data were filtered at 2 kHz. The patch potential for each record is shown above the trace.

effects of a high glucose concentration (20 mM). Mislner et al. (1986) showed that the channel was completely blocked by concentrations between 5 and 10 mM. In the upper part of Fig. 2, the triangles represent the normalized change in channel activity, measured during successive 15-s intervals, in response to the addition of glucose (see figure legend for the definition of normalized change). The initial effect of glucose was an increase in channel activity, followed by a rapid decrease that reached a steady state level within 3 min.

The circles in Fig. 2 represent the normalized change in the single-channel current amplitude measured during consecutive 15-s intervals. We consistently observed (in 12 cells) that after the addition of glucose (>0.625 mM), the channel cur-

rent amplitude increased for up to 1 min and then decreased by a much greater amount. In the case illustrated in Fig. 2, the increase was $\sim 3.2\%$ and the reduction was 24% of the control value. Assuming that this results from changes in cell membrane potential, the magnitude of the hyperpolarization and depolarization can be calculated to be ~ 2.5 and 18.6 mV, respectively, for a channel conductance of 55 pS.

Another important result in Fig. 2 is that the change in the channel current amplitude occurred only when the channel activity decreased to $< 25\%$ of its control value. Qualitatively similar data were observed in seven separate experiments. This

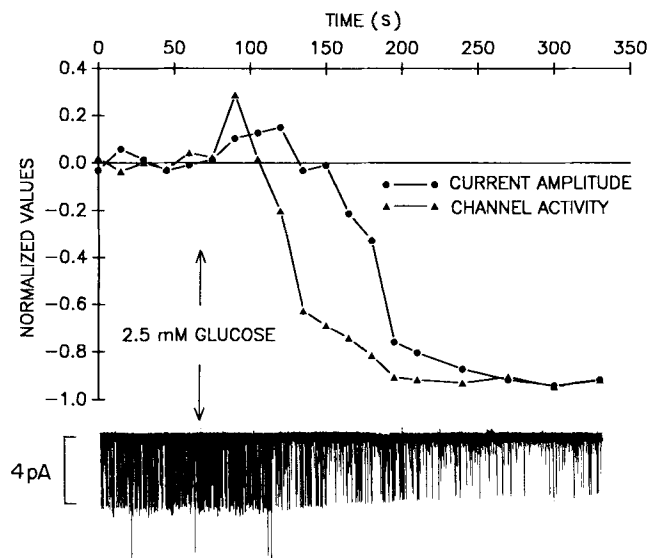


FIGURE 2. The dynamic response of the K(ATP) channel to glucose. In this experiment using a cell-attached patch, the data were digitized in 15-s blocks (sampling frequency, 5 kHz) and the channel activity and current amplitude were measured. The data for each parameter were normalized with respect to the final steady state response to glucose and plotted beneath the data on a common time scale. The initial membrane potential was estimated from the zero-current pipette potential as -75 mV, from which a channel conductance of 55 pS was calculated. From the decrease in current amplitude (from 4.1 to 3.1 pA), the membrane potential in the presence of glucose was calculated to be -56.4 mV.

observation can be predicted given a P_{Na}/P_K ratio of ~ 0.01 in the absence of glucose (Ribalet and Beigelman, 1982), necessitating a large decrease in P_K before P_{Na} becomes sufficiently significant to measurably alter the membrane potential.

The glucose dose dependence of the K(ATP) channel. It has been demonstrated that the K(ATP) channel is blocked in the intact rat β cell by the application of 20 mM glucose (Ashcroft et al., 1984; Rorsman and Trube, 1985) or by 5–10 mM glucose (Misler et al., 1986). Fig. 3 A illustrates a summary of the experiments carried out to examine the glucose sensitivity of the K(ATP) channel in RINm5F cells. From this figure, it is evident that the channel is extremely sensitive to glucose, reaching 50%

inhibition at ~ 0.5 mM glucose. However, referring to the preceding figure, where it was shown that a significant reduction in current amplitude (and therefore, by inference, a reduction in the membrane potential) did not occur until the channel activity was reduced by $\sim 75\%$, the data in Fig. 3 suggest that the depolarization of the RINm5F cell will commence at a mean glucose concentration close to 1.25 mM and will approach its maximal value at 5 mM glucose.

Fig. 3 *B* illustrates data from one of the experiments carried out to investigate the glucose sensitivity of the K(ATP) channel. In this figure, the activity represents 75, 24, and 11% of control at 0.3, 0.6, and 1.25 mM glucose, respectively. The channel current amplitude remained close to the control level at 0.3 mM glucose, whereas it

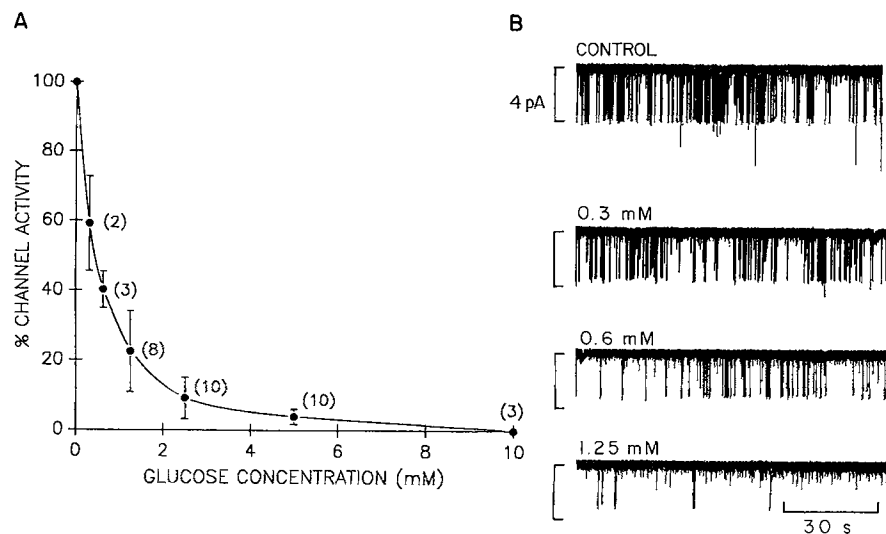


FIGURE 3. The effect of glucose on K(ATP) channel activity. (A) Glucose concentration vs. steady state channel activity in cell-attached patches. The K concentrations in the pipette and bath were 140 and 5 mM, respectively. The numbers in parentheses indicate the number of observations at each glucose concentration. The line drawn through the data points was generated by fitting a cubic spline. (B) Representative examples of steady state channel activity in the presence of the glucose concentrations indicated above each trace. Data are from a cell-attached patch experiment and were filtered at 2 kHz. Same conditions as in A.

was diminished by 20% when the activity was reduced to 24% of control in the presence of 0.6 mM glucose.

Although the activity recorded from a cell membrane patch represents a small fraction of the whole-cell channel activity, our data were consistent insofar as the relationship between the fractional decrease in channel activity and the decrease in channel current amplitude was regularly observed. This diminution of the current amplitude and its relation to channel activity was also observed with the other inhibitors of K(ATP) channel activity listed in Table I.

TABLE I
Effects of Initiators and Potentiators on the Percentage of Channel Activity of the K(ATP) and Maxi-K(V) Channels

Initiators (mM)	Channel	
	K(ATP)	Maxi-K(V)
Glucose		
0.3125	59.2 ± 13.6 (2)	—
0.625	40.1 ± 5.25 (3)	—
1.25	22.5 ± 11.6 (8)	—
2.5	9.2 ± 5.9 (10)	74.75 ± 6.6 (2)
5.0	3.91 ± 2.07 (10)	42.18 ± 10.8 (7)
10.0	0 ± 0 (3)	16.3 ± 5.07 (9)
12.5	—	9.0 (1)
15.0	—	4.25 ± 6.01 (2)
20.0	—	0 ± 0 (4)
Mannose		
1.25	3.5 ± 3.5 (2)	—
2.5	1.5 ± 1.5 (2)	—
5.0	0 ± 0 (2)	—
2-Ketoisocaproate		
1.25	11.5 ± 4.2	—
2.5	2.9 ± 1.7	—
Leucine		
10.0	—	47.3 (1)
20.0	—	5.25 ± 5.25 (2)
Potentiators		
Lactate		
10.0	87.07 ± 12.78 (2)	42.0 (1)
20	57.7 (1)	27.0 (1)
Arginine		
10.0	311.6 ± 97.4 (2)	66.0 (1)
20.0	1,159.5 ± 526 (2)	27.5 ± 5.1 (4)
TPA		
0.25 μM	1,390 ± 639 (4)	15.5 ± 13.75 (6)

To obtain the values in the table, the percentage of channel activity for each experiment was normalized with respect to the value measured in the unstimulated cell, and then the mean and standard deviation were calculated from the number of experiments given in parentheses.

Identification and Glucose Sensitivity of the Maxi-K(V) Channel

The silent phase between bursts of action potentials may result from periodic activation of a K permeability, possibly sensitive to voltage, intracellular free Ca, and metabolites, or inactivation of Ca channels by voltage and Ca itself. The intracellular accumulation of Ca ions mediated by spike activity would be responsible in either case for the periodic hyperpolarization of the cell. If a K permeability is indeed involved in the process of burst elongation and the concomitant silent phase short-

ening seen when the glucose concentration is elevated above 5–8 mM (within the stimulatory range), one reasonable proposal is that glucose may inhibit this K permeability.

Conductance and kinetic properties of the maxi-K(V) channel in cell-attached patches.

A problem encountered in studying the modulation of this channel by glucose in intact cells is that the channel is rarely active at rest, since the cells are hyperpolarized. This problem was overcome by depolarizing the cell membrane potential by addition of 30 mM K to the bath. This method was preferred over patch depolarization by changing the pipette potential since it has the advantage that it minimizes the depolarizing effect of glucose upon the cell and additionally abolishes the action potentials routinely observed in the presence of glucose. With 30 mM K, the glu-

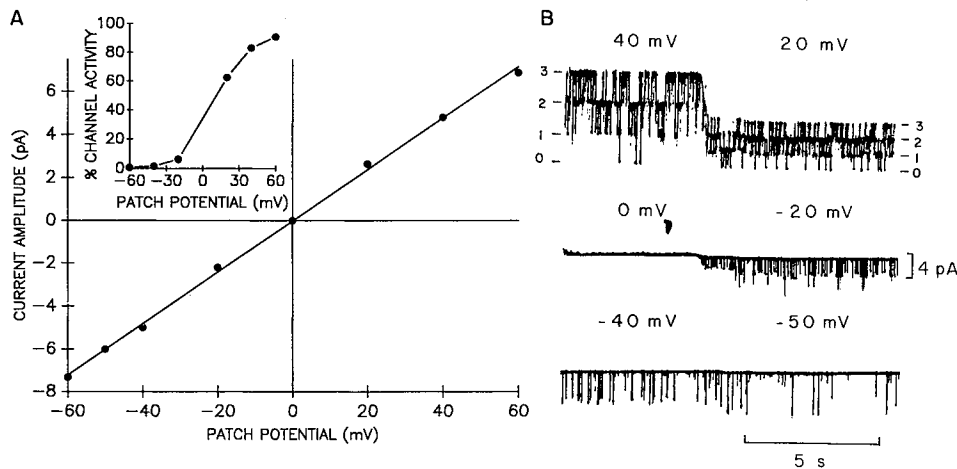


FIGURE 4. The effect of patch potential on the maxi-K(V) channel. (A) Single-channel current as a function of voltage in a cell-attached patch with 140 mM K in the pipette and 30 mM K in the bath. The membrane potential was -40 mV, estimated from the zero-current potential. The slope conductance was 120 pS. The inset shows the percentage of channel activity in the same patch as a function of patch potential. (B) Representative examples of channel current recordings from the same experiment as illustrated in Fig. 2 A. The downward deflections of the trace indicate inward current. The data were filtered at 2 kHz. The patch potential for each record is shown above the trace.

cose-induced depolarization was reduced by $\sim 65\%$. The depolarizing effect of glucose could have been avoided altogether by using a higher K concentration (60 mM K depolarizes the membrane to the peak of the action potential, in which case adding glucose has almost no effect on the membrane potential [Atwater et al., 1978]). However, we often observed that strong depolarization of the patch (to -20 mV and above) made it almost impossible to regulate the channel with glucose or any other modulator.

Fig. 4 A shows the conductive properties of the maxi-K(V) channel, where channel current is plotted as a function of patch potential ($V_m - V_p$). In this case, V_m was estimated to be -40 mV with 30 mM extracellular K. The I - V relationship is linear

for values of patch potential between -60 and 60 mV. The slope conductance in this case was ~ 120 pS, an average value of $136 \text{ pS} \pm 8.75$ having been estimated from measurements in nine patches. The data in Fig. 4 B show the strong voltage dependence of the channel activity. A low activity is seen at hyperpolarized potentials, characterized by infrequent appearances of a single current level, which evolves into almost constant activity on three current levels when the patch is depolarized. The inset to Fig. 4 A summarizes the voltage dependence of the channel activity expressed as percentage channel activity vs. patch potential. The sigmoid curve is typical of this channel with minimum activity at a patch potential at -30 mV and maximum activity at $+60$ mV.

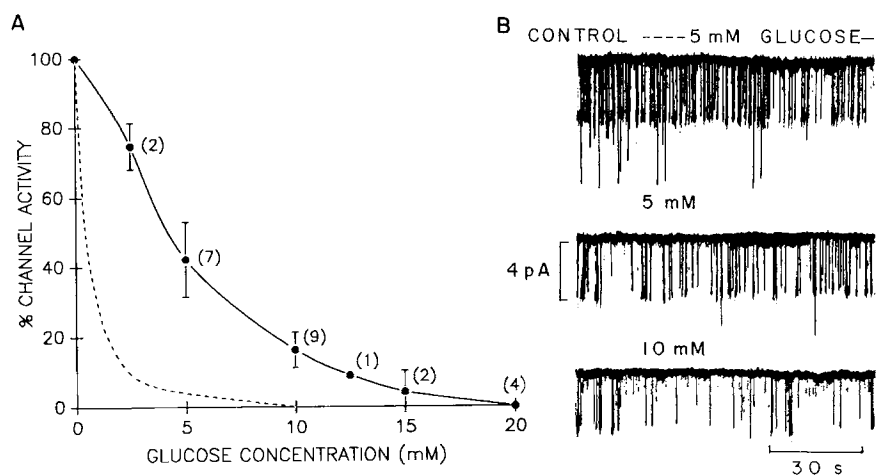


FIGURE 5. The effect of glucose on maxi-K(V) channel activity. (A) Glucose concentration vs. steady state channel activity in cell-attached patches. The K concentrations in the pipette and bath were 140 and 30 mM, respectively. The numbers in parentheses indicate the number of observations at each glucose concentration. The continuous curve through the data points was fitted using a cubic spline. For comparison, the dashed line repeats the data from Fig. 3 A, illustrating the concentration dependence of the blocking of the K(ATP) channel. (B) Representative examples of steady state channel activity in the presence of the glucose concentrations indicated above each trace. Data are from a cell-attached patch experiment and were filtered at 2 kHz. Same conditions as in A.

The glucose dose dependence of the maxi-K(V) channel. Fig. 5 A shows a summary of the normalized channel activity as a function of glucose concentration. Fig. 5 B illustrates typical channel activity recorded at various glucose concentrations. The graph shows that the channel activity was reduced to 42.5% at 5 mM glucose; complete blockage occurred only at 20 mM, a result that supports the hypothesis that the maxi-K(V) channel plays a role in burst pattern regulation. The dashed line in Fig. 5 A, representing the glucose dose-response curve for the K(ATP) channel shown in Fig. 3 A, is included for comparison of the effect of glucose on the two channels.

The Influence on the Two K Channels of Agonists That Initiate and Potentiate β Cell Activity in Normal Islets

The preceding studies illustrate that glucose influences the K(ATP) channel and the maxi-K(V) channel in two distinct concentration ranges. Consideration of these observations in conjunction with published studies of β cell membrane potential led us to propose that the roles of the two K channels may be associated with the different phases of the glucose-induced electrical activity. Thus, the K(ATP) channel functions to control the membrane potential in the range between the resting level and the threshold for bursting activity, while the maxi-K(V) channel controls the dynamics of the burst pattern by modulation of the membrane potential in the range between the silent phase and the burst plateau.

To investigate these proposals, we took advantage of the fact that previous studies on normal islets have determined that metabolic substrates can be divided into two categories: the initiators, which provoke insulin secretion and electrical activity when present alone, and the potentiators, which do not provoke the secretory response alone, but increase secretion in the presence of an initiator (Ashcroft, 1981). We hypothesized that this division of substrates into initiators and potentiators may reflect their abilities to modulate the activity of the two K channels. Therefore, (a) initiators but not potentiators have the ability to block K(ATP) channel activity and facilitate depolarization, and (b) both initiators and potentiators block maxi-K(V) channel activity in the concentration ranges associated with the secretory response. Accordingly, we tested the influence of a number of known initiators and potentiators.

The effect of the initiator glucose on the function of the two channels has been well illustrated in the preceding sections of this study. In addition, we have shown that the hexose mannose and 2-ketoisocaproate, the first breakdown product of leucine, both block the K(ATP) channel, achieving >95% blockage at 2.5 mM. The initiator leucine was used to further demonstrate the blocking effect of initiators on the maxi-K(V) channel, reaching >90% blockage at 20 mM (see Table I). The effects of the potentiating amino acid arginine and the phorbol ester TPA are described below.

The effect of arginine on the two K channels. Fig. 6 shows the effect of arginine on the activity of the two channels. A concentration of 20 mM was used, since this gives a large potentiating effect to glucose-induced electrical activity in intact islets (Beigelman et al., 1977). The effect of arginine on the maxi-K(V) channel is shown in Fig. 6 A. The addition of arginine was followed within 1 min by a decrease in channel activity, which reached a minimum value of 19% of control within 3 min. The return of control activity was considerably more rapid, occurring within the first minute of arginine removal.

By contrast, Fig. 6 B shows that the addition of arginine did not decrease K(ATP) channel activity, but after an interval provoked an increase that continued even after the removal of the amino acid. The stimulatory effect of arginine on the K(ATP) channel did not alter the concentration dependence of the glucose blockage of the channel (data not shown).

The effect of TPA on the two K channels. All the initiators of insulin release, such as glucose, mannose, leucine, and 2-ketoisocaproate, not only reduce K(ATP) chan-

nel activity, probably by elevating the intracellular ATP level, but also affect phospholipid turnover, causing an increase in the diacylglycerol level (Best et al., 1984). It has recently been demonstrated that diacylglycerol has an inhibitory effect on K channel activity in a number of different secretory cell types (see review by Ozawa and Sand, 1986). The influence of diacylglycerol is believed to occur via activation of protein kinase C and subsequent phosphorylation of specific target proteins. This finding led us to hypothesize that maxi-K(V) channel inhibition, achieved via activation of protein kinase C by diacylglycerol, may represent an intermediary step in the coupling between glucose metabolism and insulin secretion.

Of the several agents that modify the β cell secretory process via protein kinase C activation, one of the most potent is the phorbol ester TPA, which is a membrane-permeant, slowly catabolized substitute for diacylglycerol (Kaczmarek, 1986). The effect of TPA on the activity of the two K channels was investigated.

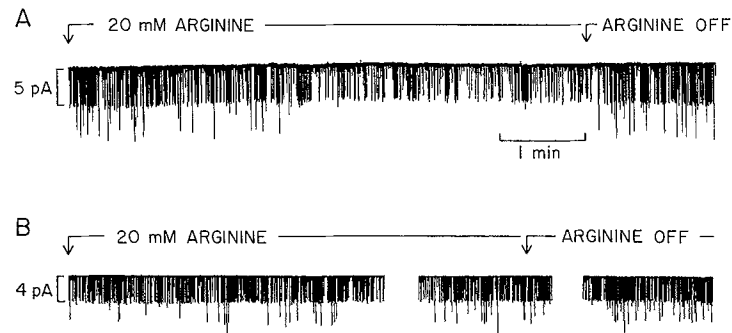


FIGURE 6. The effect of arginine on K channel activity. Arginine was perfused through the chamber at 20 mM after adjustment of the solution pH to 7.2 (A) The maxi-K(V) channel. Addition of arginine was followed within 1 min by a reduction in channel activity, which reached a steady state within 4 min. Within 1 min of return to control solution, the effect of arginine was reversed. (B) The K(ATP) channel. Addition of arginine provoked a slow increase in the level of activity of the K(ATP) channel, which continued even after removal of the amino acid. Two 3-min sections of the record have been omitted.

TPA was applied to the cell in the presence of 30 mM K. Fig. 7 A shows the result of one such experiment where the beginning of the trace coincides with the completion of the addition of 0.25 μ M TPA. This concentration of the drug was chosen because it stimulates insulin release from the normal rat β cell (Harrison et al., 1984) as well as from RINm5F cells (Hutton et al., 1984). It can be seen that maxi-K(V) channel activity was rapidly reduced by TPA and further that the effect of the drug was readily reversible upon its removal.

Since neither TPA nor diacylglycerol has a significant effect on insulin release in normal cells when glucose is absent (Harrison et al., 1984), it seems reasonable to propose that activation of protein kinase C by TPA and the ensuing phosphorylation are mechanisms primarily associated with the mode of action of agonists that function as potentiators. On the basis of this proposal, it would be expected that TPA would not reduce the activity of the K(ATP) channel.

The results of an experiment in which the cell was exposed to 0.25 μM TPA are shown in Fig. 7 *B*. The addition of TPA was followed by a slow increase in the activity of the K(ATP) channel, eventually reaching 650% of the control channel activity. The effect of TPA was poorly reversible if the drug was simply removed. However, as shown in the figure, the addition of glucose resulted in blocking of the channel. This suggests that the effect of TPA on the K(ATP) channel does not result from interference with the basic modulatory system of channel activity.

In addition to arginine and TPA, the triose lactate was used to investigate the effect of potentiators on the activity of the two channels. As shown in Table I, lactate achieved >70% blockage of the maxi-K(V) channel at 20 mM, while the same concentration caused an ~40% blockage of the K(ATP) channel with no concomi-

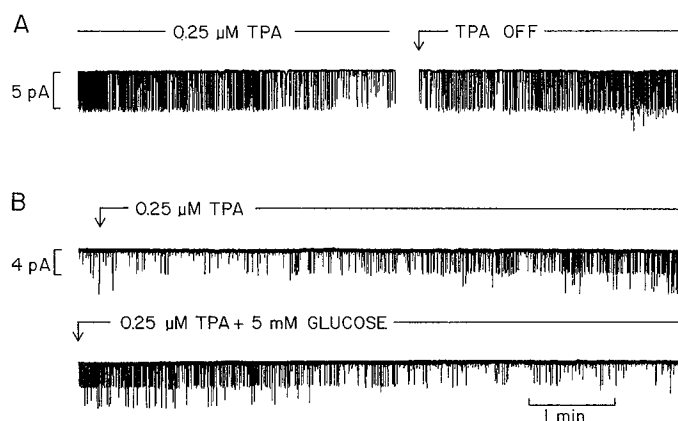


FIGURE 7. The effect of TPA on K channel activity. (A) The maxi-K(V) channel. Addition of 250 nM TPA to the bath was completed at the start of the record; its effect on channel activity occurred during the following 3 min. 2 min of steady state activity before the removal of TPA has been omitted. The recovery from the effect of TPA was rapid, being complete in <3 min. (B) The K(ATP) channel. The effect of 250 nM TPA on the K(ATP) channel was progressive. In other experiments where its action was not interrupted by the addition of glucose, TPA provoked increases in percentage of channel activity in excess of 1,000% of control. In this experiment, however, it can be seen that the effect of glucose in blocking the channel was not impaired by TPA.

tant change in channel amplitude, which suggests that no change in membrane potential occurred. Table I shows a summary of the effect of several initiators and potentiators on the normalized activity of both K channels.

DISCUSSION

Macroscopic aspects of β cell electrophysiology and their association with secretory behavior have been well described previously (Dean et al., 1975). Patch-clamp studies have recently added information concerning microscopic (i.e., single-channel) details of islet cell membrane electrical activity (for review, see Petersen and Findlay, 1987). In this study, we have described two K channels in RINm5F cells that differ in their conductance, kinetics, and glucose sensitivity. We have proposed a relation-

ship between the mode of action of initiators and potentiators of insulin secretion in normal cells and their different effects on the two channels. The results of experiments using the potentiator TPA have indicated a possible link between glucose metabolism and K channel modulation in addition to the direct effect of ATP on the K(ATP) channel.

The better-defined, though more recently discovered, β cell membrane K channel is the K(ATP) channel (Petersen and Findlay, 1987). The mean conductance we calculated for the K(ATP) channel was 55.1 ± 5.2 pS. This is in good agreement with the majority of previous measurements of K(ATP) channel conductance in rat and mouse β cells as well as in RINm5F cells. The exhibition of inward rectification and the absence of voltage sensitivity are also in agreement with previous reports (Cook and Hales, 1984; Rorsman and Trube, 1985; Kakei et al., 1985; Mislner et al., 1986; Dunne et al., 1986). Since the K(ATP) channel is blocked by direct application of ATP in isolated inside-out β cell membrane patches, we assume, as have others (Rorsman and Trube, 1985; Kakei et al., 1985; Mislner et al., 1986; Dunne et al., 1986), that the effect of glucose is mediated via its influence on cellular ATP levels.

In the present study, we have defined the glucose concentration vs. channel activity profile, showing that channel activity is reduced by 50% of its control value at 0.5 mM glucose and the channel is almost fully inactivated at 5 mM glucose. Recent studies on the rat β cell indicate a similar glucose sensitivity of the K(ATP) channel, with half-maximum blockage at close to 1.25 mM glucose and complete blockage at ~ 5 mM (Ashcroft et al., 1986; Mislner et al., 1986). This result is significant since it demonstrates that, in RINm5F cells, the absence of an insulin secretory response to glucose is not a consequence of a low K(ATP) channel sensitivity to glucose.

On the basis of the assumption that the K concentration in the pipette is close to that of the intracellular medium, we estimated from the zero-current potential that the resting membrane potential was close to -71 mV. This value is similar to that reported in patch-clamp studies by Dunne et al. (1986) for the RINm5F cell and by Ashcroft et al. (1984) for the rat β cell and to the mouse β cell resting potential measured using microelectrodes (Meissner et al., 1978). The observations of the reduction of the channel current amplitude after application of glucose and other secretagogues are independent of this estimate. This current decrease indicates a reduction in the driving force that can almost certainly be attributed to a change in the membrane potential. Two important findings come from these results. One is the confirmation that the RINm5F cell does indeed undergo depolarization in response to glucose, permitting rejection of the hypothesis that the basis for the lack of an insulin secretory response to glucose in the RINm5F cell resides in the inability of the sugar to initiate depolarization (Rorsman et al., 1983). The other important finding is the illustration of the necessity for a $>70\%$ decrease in channel activity before the cell can depolarize. Referring to the glucose vs. percentage of channel activity plot of Fig. 3 A, one can estimate the mean glucose concentration threshold for depolarization to be close to 1.25 mM.

The dynamics of the change of membrane potential, as inferred from the measurement of the single-channel current amplitude (see Fig. 2), well reflect the membrane potential response after glucose addition measured in microelectrode studies

of mouse islet β cells. The initial effect of glucose on the isolated RINm5F cell is an increase in current amplitude lasting ~ 1 min, which is followed by a decline that reaches a steady state after ~ 3 min. This timing is entirely consistent with the observations of Atwater et al. (1978), who reported that in mouse islet β cells, an initial 3–5-mV hyperpolarization, accompanied by a decrease in cell input resistance lasting ~ 1 min, occurred upon glucose addition. This was followed by a depolarization averaging 17% of the resting potential and a concomitant rise in cell input resistance leading to the initiation of electrical activity after another 2 min.

The magnitude of the current change after application of glucose to the RINm5F cell permits estimation of the changes in membrane potential. The transient increase and subsequent decrease in the channel current amplitude observed in the presence of 5 mM glucose were in the ranges 3–4 and 20–30%, respectively, which suggests that the initiator-induced changes in membrane potential amount to an early hyperpolarization and a succeeding depolarization of 2.1–2.8 and 14–21 mV (assuming a channel conductance of ~ 55 pS). Similar observations of depolarization have been reported by Dunne et al. (1986) using glyceraldehyde in RINm5F cells and by Ashcroft et al. (1987) using 2-ketoisocaproate in mouse β cells.

These data, along with the previous observations, lend weight to the hypothesis that the role of the K(ATP) channel is limited to modulation of the membrane potential between the resting potential and the threshold potential for the activation of the electrical burst pattern. This hypothesis is further supported by the finding that intracellular ATP reaches 80% of its maximum value when extracellular glucose is raised to 5 mM, with only a minor further increment in the secretory glucose range (Ashcroft et al., 1973; Malaisse et al., 1979).

Fig. 4 illustrates the kinetics and conductance properties of the maxi-K(V) channel. It can be seen that this channel has very similar kinetics to the Ca and voltage-dependent K channel [K(Ca,V)] channel reported for isolated patches of both rat β cells (Cook et al., 1984; Findlay et al., 1985*a, b*) and RINm5F cells (Findlay et al., 1985*c*). However, the conductance in the cell-attached patch is significantly smaller, 136 ± 8.75 pS ($n = 9$) vs. > 200 pS. Since we did not observe a conductance approaching 200 pS in cell-attached patch studies, although the activity of the larger glucose-sensitive K channel could be stimulated by treatments that elevate intracellular Ca (application of DNP or the Ca ionophore A23187; results not shown), it is possible that the conductance of the K(Ca,V) channel is modified by elements present in the intracellular milieu. However, in view of the discrepancy between the conductance values, it is preferable to refer to this channel using a different name, e.g., maxi-K(V), thus allowing for the possibility that it consists of a different molecular entity from K(Ca,V).

The data in Fig. 5 demonstrate that, as with the K(ATP) channel, the modulation of the maxi-K(V) channel is influenced by glucose. It is of interest that the maxi-K(V) channel activity is blocked by $> 50\%$ at 5 mM glucose, a concentration that in the normal β cell does not cause a significant increase of insulin secretion. It must be borne in mind, however, that the maxi-K(V) channel is not active at hyperpolarized potentials (see inset to Fig. 4), so this modulation of activity would not normally be manifested. However, the channel sensitivity to glucose extends over the secretory range for the normal β cell, which suggests that there may be some causal-

ity between the progressive deactivation of the maxi-K(V) channel and the shortening of the silent phases between bursts of electrical activity that occurs as glucose is increased. Its voltage sensitivity would keep this channel from regulating the membrane potential at rest.

In the preceding discussion, we have demonstrated that the roles of the two K channels may be differentiated by the ranges of glucose to which they respond. The K(ATP) channel is significant in the glucose range 0–5 mM, exerting its effect on the membrane potential between 1.25 and 5 mM, while the maxi-K(V) channel, which is inactive until the cell membrane is depolarized up to the threshold for Ca current activation, becomes prominent between 5 and 20 mM glucose.

Although glucose is considered to be the major physiological modulator of insulin secretion, many other agents—metabolic substrates, hormones, and neurotransmitters—may influence insulin secretion. We have used agents that initiate and potentiate insulin secretion in normal cells to test the hypothesis that the division of substrates into these categories reflects their abilities to effect in the former case blockage of both the K channels and in the latter case modulation of the activity of the maxi-K(V) channel alone. The data obtained with glucose demonstrate that the primary β cell initiator follows this pattern. The initiating amino acid leucine or its first breakdown product, 2-ketoisocaproate, gave similar results with blockage of both channels. All of the potentiators blocked the maxi-K(V) channel, 20 mM of both lactate and arginine having the equivalent blocking effect of 7.5 mM glucose, while TPA blocked the channel with an effect equivalent to 11 mM glucose (measured from Fig. 5). In investigations of the effect of potentiators on the K(ATP) channel, arginine and TPA produced unexpected results, each causing activation of the channel. This clearly would not result in depolarization. While the third potentiator, lactate, caused a >40% decrease in channel activity, this would be insufficient to provoke depolarization (see Fig. 3). Despite their stimulatory effects on the K(ATP) channel, neither TPA nor arginine prevented the channel blockage induced by glucose. The results obtained in these experiments are supportive of the stated hypothesis that the division into initiators and potentiators is reflected in the different abilities of these agents to influence the activity of the K(ATP) and the maxi-K(V) channel.

If these channel modulations also occur in the normal β cell, it can be seen that control of channel activity may represent an important locus in the glucose homeostasis of the whole animal. The maxi-K(V) channel can modulate secretion only if K(ATP) channel activity is inhibited; otherwise, when the circulating glucose concentration is insufficient to maintain its inhibition, the K(ATP) channel acts to prevent secretion, independently of the presence of potentiators. Activation of the K(ATP) channel as a result of low circulating glucose would cause the membrane to hyperpolarize such that the cell would be in a state of secretory refractoriness. Under such conditions, the potentiators would not influence secretory activity, thereby preventing further reduction in the glucose level.

The interpretation of the effect of TPA on cellular events is based upon its ability to replace diacylglycerol in the stimulation of protein kinase C, the latter exerting its effect via phosphorylation of specific target proteins. Phospholipid turnover and the ensuing production of diacylglycerol is stimulated by insulinotropic agents such

as glucose, mannose, leucine, 2-ketoisocaproate (Best et al., 1984), and perhaps arginine (Fex and Lernmark, 1972). The observation that maxi-K(V) channel activity is blocked by TPA therefore suggests that its modulation by glucose may be in part related to phosphorylation by protein kinase C. Data published in microelectrode studies of the effect of TPA in the mouse β cell support the results obtained at the single-channel level in the RINm5F cell. Pace and Goldsmith (1985) observed that application of TPA had no effect on the membrane potential when present alone, but caused an enhancement of the electrical activity when glucose was present. The inhibitory effect of the phorbol ester on the maxi-K(V) channel, but not the K(ATP) channel, would cause such an enhancement of the electrical activity in the presence of glucose, while having no effect on the resting membrane potential.

The finding that maneuvers that elevate intracellular cAMP (addition of forskolin or isobutylmethylxanthine) abolish the silent phases between bursts (Eddlestone et al., 1985) suggests that phosphorylation resulting from a cAMP-induced increase in protein kinase A activity may also cause a decrease in maxi-K(V) channel activity. Of the paracrine hormones, glucagon potentiates insulin secretion and also stimulates adenylate cyclase, while somatostatin inhibits secretion and inhibits adenylate cyclase. These observations suggest that phosphorylation resulting from protein kinase A activation may represent an alternative pathway for channel modulation. Although there is now evidence that the channel proteins may be directly phosphorylated (Ewald et al., 1985), it is clear that a number of intermediate steps may lie between phosphorylation and channel regulation.

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