INTRODUCTION

The technique of electrically isolating a patch of membrane using a glass pipette and studying the activity of the ion channels (Hamill et al., 1981; Miller, 1986; Neher et al., 1978) differs from classical electrophysiological methods by permitting direct observation of ion currents through channel proteins in their native environment. The currents can be amplified, recorded and analyzed in terms of single-channel parameters such as conductance, selectivity and pharmacology. The channel proteins can be isolated, purified and incorporated into planar lipid bilayers (Miller, 1986) to further facilitate study, and channel structure can be explored at the molecular and atomic level using the Xenopus oocyte expression system (Claudio et al., 1987; Gundersen et al., 1983; Methfessel et al., 1986; Noda et al., 1984; Snutch, 1988).

K⁺ channels play a critical role in determining the resting membrane potential, and the time course and amplitude of membrane electrical changes in nearly all cells. Evidence is rapidly accumulating that membrane potential is an important trigger for activity even in the cells classically considered as nonexcitable. K⁺ channels vary widely in their kinetics, gating, pharmacology, single channel parameters and other properties. A single cell may have many different types of K channels, and many cells may have similar populations of K channels. The number of different types of K channels that have been identified is still increasing and may exceed 100.

The K⁺ channels observed in epithelial cells include voltage-dependent K⁺ channels (mainly delayed rectifiers, which are channels that conduct outward current after a brief delay following membrane depolarization), Ca²⁺-activated K⁺ channels, and K⁺ channels that are modulated by ATP or GTP-β binding proteins. A number of reviews of K⁺ channels in excitable cells have been published (Armstrong, 1975; Duty and Weston, 1988; Hille, 1992; Hodgkin and Huxley, 1952; Kolb, 1990; Latorre, 1986; Latorre and Miller, 1983; Petersen and Maruyama, 1984; Pongs, 1989; Robertson and

Abstract—The broad array of K⁺ channels in epithelial cells includes voltage-dependent (mainly outward) and Ca²⁺-activated channels, and K⁺ channels modulated by adenosine triphosphate (ATP). Voltage-dependent K⁺ channels mediate Na⁺/K⁺ absorption and secretion; typically, they are found in the basolateral membrane and exhibit burst activity. Ca²⁺-activated K (Ca²⁺/K⁺) channels regulate activity by decreasing Ca²⁺ influx via voltage-gated Ca²⁺ channels. Ca²⁺/K⁺ channels exhibit conductances of 4-300 pS, and have a low open probability (< 10) at the level of the resting membrane potential. ATP-sensitive channels have been observed mainly in insulin-secreting pancreatic β-cells and in the urinary tract, where the open state is rapidly closed by ATP. The channels are voltage-dependent, exhibit burst activity, and, in the case of the urinary-tract cells, are Ca²⁺ dependent. Chemical compounds that selectively open or block K⁺ channels have been exploited to characterize channels found in different cells, but no opener or blocker has been found that specifically affects only one type of K⁺ channel. Specialized model systems and recombinant techniques have led to a general understanding of the structure of K⁺ channels, but many important details remain to be determined.

Keywords—Potassium channels, Epithelial cells

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Hepatocytes
Pancreatic ß
Choroid plexus
Lens
Renal
Airway

voltage changes toward depolarization increased the 1988; Rudy, 1988), and displayed bursting behavior; and Miller, 1983; McCann and Welsh, (Christensen and Zeuthen, 1987; Kohlb, 1990; Latorre and Miller, 1992; Sauve et al., 1986, 1987; Van Driessche and Zeiske. 1985; Wang et al., 1992).

**VOLTAGE-DEPENDENT CHANNELS**

Voltage-dependent K⁺ channels open and close in response to changes of membrane voltage (Table 1). Outward-going K⁺ channels are activated by membrane depolarization which increases open-time probability; the channels carry outward K⁺ currents with a time constant of hundreds of milliseconds. In some types of epithelial cells, including the renal proximal tubule and the diluting segment (Bleich et al., 1990; Wang et al., 1992), distal tubule and collecting duct (Lang and Rehwald, 1992; Wang et al., 1992), the K⁺ channels are found in both basolateral and apical membranes, where they are responsible for Na⁺/K⁺ absorption and secretion. But voltage-dependent K⁺ channels are found mainly in the basolateral membrane. Except in colonic epithelial cells (Dawson, 1991); they occur in epithelial cells in many tissues including the lung, liver, kidney and pancreas (Table 1). The channels were highly selective for K⁺ as determined from measurements of the reversal potential (Christensen and Zeuthen, 1987; Kolb, 1990; Latorre and Miller, 1983; McCann and Welsh, 1990; Rae et al., 1988, 1988), and displayed bursting behavior; voltage changes toward depolarization increased the burst frequencies (Gogelein and Greger, 1987; Rae et al., 1988; Wang et al., 1990). This oscillatory behavior has not been explained yet, but probably results from the oscillations in intracellular Ca²⁺ levels.

The K⁺ channel blockers, 4-aminopyridine (4-AP) and tetraethylammonium (TEA) block outward-going K⁺ channels (Hille, 1992); 4-AP usually blocks equally well when applied inside the cell, but TEA blocking effects differed when the agent was applied internally (Latorre and Miller, 1983), suggesting two different binding sites and mechanisms of action. TEA is generally more effective when applied externally (Adams and Nonner, 1990; Armstrong, 1975; Cook and Quast, 1990). Ba²⁺ is a strong blocker (Hille, 1992; Latorre and Miller, 1983; Wang et al., 1990); it interacts with the same sites as K⁺ because the two ions have similar nonhydrated diameters (0.270 and 0.267 nm, respectively). Cs⁺ can block delayed rectifier channels when applied internally or externally (Hille, 1992) because K⁺ channels are narrow (0.3 nm) and large ions like Cs⁺ (0.338 nm) occlude the channel. Generally, externally applied Cs blocks inward but not outward K⁺ currents, whereas the opposite is true for internal Cs⁺ (Latorre and Miller, 1983).

Inward rectifiers (channels that pass inward K⁺ current following hyperpolarization) have been described in kidney and liver cells (Friedrich et al., 1988; Gogelein and Greger, 1987; Henderson et al., 1989; Hunter, 1988; Hunter et al., 1988; Parent et al., 1988), but it is difficult to assess their physiological function in these cells. In general, inward rectifiers take part in processes like egg fertilization, phagocytosis, chemotaxis, and restoration of the resting potential. Inward

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<tr>
<th>Epithelial Cell</th>
<th>Activation (mV)</th>
<th>Inactivation (msec)</th>
<th>Blockers</th>
<th>Single-Channel Properties</th>
<th>Reference</th>
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<tr>
<td>Airway</td>
<td>-80 to -40</td>
<td>100</td>
<td>TEA, 4-AP ChTx (10 μM)</td>
<td>Burst activity</td>
<td>22-25 pS</td>
</tr>
<tr>
<td>Respiratory</td>
<td>-60 to 0</td>
<td>00-4000</td>
<td>TEA, lidocaine, quinidine (1 mM), Ba²⁺</td>
<td>Burst activity</td>
<td>120-230 pS</td>
</tr>
<tr>
<td>Renal</td>
<td>-100 to -50</td>
<td>100-30,000</td>
<td>TEA (10 mM), 4-AP (5 mM), Ba²⁺ (1 mM), quinidine</td>
<td>Burst activity</td>
<td>21-50 pS</td>
</tr>
<tr>
<td>Lens</td>
<td>-90 to -30</td>
<td>10-20</td>
<td>TEA, quinine, Ba²⁺, Cs²⁺</td>
<td>Multilevel conductance</td>
<td>10-150 pS</td>
</tr>
<tr>
<td>Choroid plexus</td>
<td>-20</td>
<td>60-100</td>
<td>pH &lt; 7.4</td>
<td>Burst activity</td>
<td>224 pS</td>
</tr>
<tr>
<td>Mammary</td>
<td>-40 to -30</td>
<td>100</td>
<td>TEA (50 mM), Ba²⁺ (0.1 nM)</td>
<td>Flicker closing</td>
<td>12 pS</td>
</tr>
<tr>
<td>Pancreatic ß-cells</td>
<td>-40</td>
<td>100-1000</td>
<td>TEA (4-AP)</td>
<td>Burst activity</td>
<td>20-250 pS</td>
</tr>
<tr>
<td>Hepatocytes</td>
<td>-100 to -40</td>
<td>150-500</td>
<td>Ba²⁺</td>
<td>Burst activity</td>
<td>10-30 pS</td>
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</table>
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rectifiers in nonexcitable cell membranes are blocked by TEA via a voltage-dependent mechanism (Rudy, 1988). Cs+ ions strongly block inward rectifiers in a voltage and time-dependent fashion in starfish eggs (Hagiwara et al., 1976). Na+ may block inward rectifier K+ channels at negative potentials (Ohmori, 1978). None of these channels are activated by Ca2+, even in the pM range. Inward rectifiers can be specifically blocked in a voltage-dependent manner by intracellular Mg2+, whose blocking rate constant is of the same order as the K+ transport rate through the channel (for review, see Matsuda et al., 1987; Vandenberg, 1987). Hyperpolarization beyond EK causes displacement of Mg2+ and increases the K+ inward current, while depolarization leads to steric block; in the absence of Mg2+ the I/V relationship is linear (Matsuda et al., 1987). Until now, this effect has been described only in excitable cells.

Because of the similarities between inward and outward rectifiers in unitary conductance, ion selectivity and blocking behavior, it seems reasonable to expect similarities in their basic architecture (Hille, 1992).

**Ca2+-ACTIVATED CHANNELS**

In the class of K’-channels to be considered now (Table 2), increasing intracellular Ca2+ opens the channel, sometimes in a voltage-dependent manner. When the Ca2+ cytoplasmic concentration becomes increased, the induced K+ efflux hyperpolarizes the cell, thus decreasing Ca2+ influx via voltage-gated Ca2+ channels and thereby regulating secretion (Cook et al., 1984; Dunne et al., 1988; Findlay et al., 1985a; Kolb, 1990; Maruyama et al., 1983; Petersen and Dunne, 1985; Petersen and Maruyama, 1984). Conductance of Ca2+-activated K (Ca2+/K+) channels varied from 4300 pS; channels with values of 140-300 pS are often designated as maxi Ca2+-activated K’ channels (maxi Ca2+/K’). For most epithelial cells, Ca2+/K’ channels have a small open probability (P_o ≤ 10^-7) at the level of resting membrane potential (Christensen and Zeuthen, 1987; Kolb, 1990; Kolb et al., 1986), but P_o is voltage sensitive and can shift 10-fold for depolarizations of 10-15 mV (Kolb, 1990; Kolb et al., 1986).

Ca2+/K’ channels may be affected by Ca2+ in two different ways. Ca2+ may interact directly with the channel protein as a ligand, or it may act as a second messenger to activate a cell enzyme system which then activates the channel. Most reconstitution experiments in planar lipid bilayers show that Ca2+ can interact directly with channel proteins to effect opening of channels (Latorre et al., 1985).

Ca2+/K’ channels have been found in many secretory and nonsecretory cells (Table 2). In excitable cells, Ca2+/K’ channels regulate repetitive activity and produce long-lasting hyperpolarization; in nonexcitable cells their opening induces closing of voltage-dependent Ca2+ channels. They are strongly selective for K’ at normal physiological K concentration. The reported ion-selectivity sequence for the channel in nerve and muscle cells (Latorre and Miller, 1983) was Tl’ > K’ > Rb’ > NH4+ > Na’ > Li’; the ion-selectivity of all K’ channels is similar, although variations in relative permeabilities in some types of K’ channels have been reported (Kolb, 1990).

TEA reversibly blocks Ca2+/K’ channels in low concentrations (0.1–1.0 mM) when applied to the external membrane surface. The blocking reaction can be voltage-independent, and there is evidence that TEA binds to different sites in different Ca2+/K’ channels (McCann and Welsh, 1990; Yellen, 1987). These findings differ from observations on muscles and neurons, where TEA blockade is generally voltage-dependent.

Charybdotoxin (ChTx) (from the scorpion Leurus quinquestratus) is a specific blocker for Ca2+/K’ channels; it inhibits the channels at concentrations of 1-10 nM (Cook and Quast, 1990; Dreyer, 1990). ChTx has been shown to inhibit two types of Ca2+/K’ channels in cultured airway epithelial cells at 10 nM (McCann et al., 1990; McCann and Welsh, 1990); it blocks the channels reversibly when applied externally. Quinine, another inhibitor of the Ca2+/K’ channels, blocks maxi Ca2+/K’ channels in many epithelial cells, but it may or may not block Ca2+/K’ channels in cultured insulin-secreting cells (Findlay et al., 1985b).

In pancreatic β-cells (Cook et al., 1984), raising the intracellular pH increased the open probability of Ca2+/K’ channels, while in choroid plexus epithelial cells (Christensen and Zeuthen, 1987) it mainly affected the closed time. A possible explanation for the latter observation is that W compete with Ca2+ for binding sites in the channel protein molecule: H+ binding prevents Ca2+ binding and therefore its effect occurs only while the channel is closed. The physiological importance of the intracellular pH sensitivity is still uncertain; perhaps decreasing cytoplasmic pH as a result of metabolic processes is important in the regulation of insulin production, or in the electrogenic reabsorption in renal proximal tubule (Boron and Boulpaep, 1983; Wang et al., 1992).

The large conductances of maxi Ca2+/K’ channels yield a high signal/noise ratio in most records, thus allowing single-channel currents to be analyzed with a good time resolution. Ca2+ binding sites have been demonstrated in patched membranes and following re-
Table 2. Single-Channel Parameters of Calcium-Activated K Channels in Epithelial Cells. The Channels are Opened by Increasing Cytosolic Ca2+ Levels in a Voltage-Dependent Manner (Except Where Noted). Channels Shown to be Gated by Neurotransmitters are Indicated by Lettered Superscripts. S, E, H (Serotonin, Epinephrine, Histamine, Respectively)

<table>
<thead>
<tr>
<th>Epithelial Cell</th>
<th>Blockers</th>
<th>Single-Channel Properties</th>
<th>References</th>
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<tr>
<td>Secretory cells</td>
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<tr>
<td>Mouse parotid acinar</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Mouse salivary acinar</td>
<td></td>
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<tr>
<td>Pancreatic β</td>
<td>Ba2+ (2 mM)</td>
<td>4-300 pS</td>
<td>6, 20, 22, 41, 44, 47, 73, 85, 88</td>
</tr>
<tr>
<td>Adrenocortical</td>
<td>Cs2+ (10 mM)</td>
<td></td>
<td></td>
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<tr>
<td>Mouse lacrimal acinar</td>
<td>TEA (0.1-1 mM)</td>
<td>Subconductance states</td>
<td></td>
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<tr>
<td>Rat liver cells</td>
<td>Quinine (1 µM)</td>
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<td></td>
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<tr>
<td>Rat hepatocytes</td>
<td>Mg2+ (10^3–10^6 M)</td>
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<td>121</td>
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<td>Rat intestinal</td>
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<td>Choroid plexus</td>
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<td>Cultured mammary cells</td>
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<td>Nonsecretory cells</td>
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<td>Nephron</td>
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<tr>
<td>Rat distal collecting tubule</td>
<td>Ba2+ (100 µM-2 mM)</td>
<td>30-250 pS</td>
<td>15, 22, 31, 46, 47, 63, 64, 73, 87,</td>
</tr>
<tr>
<td>MDCK cells</td>
<td>TEA (2-10 mM)</td>
<td>Burst activity</td>
<td>88, 99, 104, 113, 114, 126, 12</td>
</tr>
<tr>
<td>Renal epithelium</td>
<td>ChTx (10 nM)</td>
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<tr>
<td>Rat cortical collecting tubule</td>
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<td>Rat proximal CCT</td>
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<tr>
<td>Cultured respiratory cells</td>
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<td>HeLa cells</td>
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<td>Lens cells</td>
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1Inward rectifier.
2Flicker behavior.
3Voltage independent.
4TEA (5 mM).
5pH ≥ 7.4.

The range of kinetic activity of Ca2+/K+ channels was well demonstrated in cultured rat myotubes (McManus and Magleby, 1988), which showed four different channel modes: normal, intermediate-open, brief-open and bursting. It was not clear whether the frequencies of appearance of these modes was dependent upon Ca2+ concentration. Analyses of open states and dwell-time distributions showed that the channels entered into a discrete state for a finite time and then rapidly passed into the fully open state (Colquhoun and Hawkes, 1983), following complicated kinetics that involved multieponential-function distributions of all open and closed states (Colquhoun and Hawkes, 1983, 1990). An analysis of channel open-time distributions based on a fractal model was reported (Liebovitch et al., 1987); the channel kinetics were described using two parameters for both the open- and closed-time distributions, but the model has not yet been applied to Ca2+/K+ channels. Al-though subconductance states of Ca2+/K+ channels have been observed (Furuya et al., 1989; Kolb et al., 1986), they have not been included in the kinetic models used to analyze channel types with well resolved subconductance states, such as Na+ and

constitution of the channels in lipid bilayers (Latorre et al., 1985). Reconstitution experiments show that Ca2+-calmodulin interaction, protein-kinase C activation and phosphorylation are not involved in Ca2+/K+ channel kinetics. The Ca2+ sensitivity of maxi K+/Ca2+ channel varied considerably in different cell types (Petersen and Maruyama, 1984); intracellular Ca2+ concentrations in the range of 10^-7 to 10^-5 were effective for channel activation (Findlay, 1984; Hodgkin and Huxley, 1952; Kolb, 1990; Kolb et al., 1986; Maruyama et al., 1983). The channel open state was characterized by the binding of one Ca2+ in native membrane experiments, while 2 or more Ca2+ were required in experiments involving reconstituted channels. A decrease of channel permeability with increasing Ca2+ concentration was observed, but no satisfactory explanation has been presented. Channel activity was also regulated by internal Mg2+; concentrations of 10^-6 to 10^-3 M (at constant Ca2+) evoked an increased channel open-probability in salivary-gland cells (McCann and Welsh, 1990) (the physiological range was 0.4-3.0 MM). Mg2+ may induce fast conformational changes in the channel.
K⁺ channels in neurons (Colquhoun and Sakmann, 1985; Fox, 1987; Geletyuk and Kazachenko, 1989; Iliev, 1989).

Channel density of Ca²⁺/K⁺ channels has been estimated for a number of different cells. For lacrimal-gland cells, the population was 50-150 channels (Trautmann and Marty, 1984); if a cell diameter of 15 µm is assumed then the density would correspond to 5-15 µm⁻²/channel. For renal epithelial cells and MCDK cells, the density corresponded to 16.9 µm²/channel (DeCoursey et al., 1985) and 5 µm²/channel (Hunter et al., 1986), respectively. In rat salivary acinar cells the density was 10 µm²/channel (Cook et al., 1984; Maruyama et al., 1983), and a density of 2.5 µm²/channel was reported in cells of the choroid plexus (Christensen and Zeuthen, 1987).

### CHANNEL MODULATION BY ATP AND G PROTEINS

ATP-sensitive K⁺ channels have been observed mainly in insulin-secreting pancreatic β-cells and in the urinary tract (Table 3), where the open state is rapidly closed by ATP. An effect of ATP on outward and inward channels was observed (Lewis et al., 1990); increasing concentration of ATP tended to reduce the channel open time. An increase in ADP concentration had the opposite effect, without any changes in the magnitude of the single-channel currents (Dunne et al., 1988). The effect of ATP on K channels is not mediated by channel phosphorylation and does not require the presence of Mg²⁺. ATP is actually more effective in the absence of Mg²⁺. The kinetics of the ATP-sensitive K⁺ channels are complex, consisting of a high open-channel probability during bursts, separated by long closed periods (Findlay and Dunne, 1986; Kakei et al., 1986). There is some evidence that one open and two closed states are necessary to produce the observed kinetics of the ATP-sensitive K⁺ channels (Kakei et al., 1986; Rorsman and Trube, 1985). Single-channel kinetics of ATP-sensitive K⁺ channels are voltage-dependent (Ashcroft et al., 1987; Kakei et al., 1986). Outward currents are associated with long open times, whereas inward currents show burst kinetics (Ashcroft et al., 1987).

ATP-sensitive K⁺ channels are permeable to Rb⁺ (Ashcroft et al., 1987), suggesting that Rb⁺ can enter the channel from the inside as easily as the K⁺, but that Rb⁺ becomes bound to the sites in the pore where K usually binds, thus blocking K⁺ currents. On average, one functional channel is present in each 3 µm² of membrane (Ashcroft et al., 1984).

The discovery that ATP-sensitive K⁺ channels are the major K⁺ channels in β-cells suggests that many pharmacological agents producing a decrease of K⁺ permeability might act as channel blockers. Patch-clamp experiments identified two sulphonylureas, tolbutamide and glibenclamide, as specific blockers of ATP-sensitive K⁺ channels (Ashcroft et al., 1987; Rorsman and Trube, 1985; Rorsman and Trube, 1990; Trube et al., 1986). Both agents are effective in nM concentrations (Rorsman and Trube, 1990). ATP-sensitive K⁺ channels are also affected by 4-AP, TEA, and quinine, when they are applied to either the external or internal membrane surface. The blockers appear to decrease the frequency of channel openings without any changes in the amplitude of single channel currents, but dose-response curves for these agents have not been reported. The channels were also blocked by a number of cations added to the internal solution (Cook and Hales, 1984); the block was voltage-dependent, which supports the idea that the ions can enter the channel conductance pathway but are unable to pass through the channel's selective filter.

The question arises whether the ATP-sensitive K⁺ channel can phosphorylate itself, or whether other membrane proteins participate in the process. If the latter is correct, the responsible protein kinase must be located close to the channel protein in the membrane.
Further experiments are required to resolve this question.

Some K channels are modulated by the GTP-binding proteins (G-proteins), resulting in changes in channel conductance over periods of seconds or even minutes. Different forms of G-proteins activate K\(^+\) channels in different tissues (Brown, 1993). In addition to direct actions on ion channel activity, G-proteins also influence channel function by activating different second-messenger cascades including cAMP-mediated protein phosphorylation and phospholipase C leading to the hydrolysis of phosphatidylinositol and release of diacylglycerol (Berridge and Irvine, 1984; Nishizuka, 1984). Both agents induce release of calcium from intracellular stores, and thus lead to activation of Ca\(^{2+}/\)K\(^+\) channels (Berridge, 1993). A third pathway involves activation of lipoxygenase metabolites of arachidonic acid through activation of phospholipase C by G-protein dependent mechanisms (Parekh et al., 1993).

There is also evidence that G proteins can connect membrane receptors with ion channels by unknown cytoplasmic pathways (Axelrod et al., 1988; Birnbaumer et al., 1990; Brown and Birnbaumer, 1988, 1990; Kubo et al., 1993a; Lewis et al., 1990). A single G-protein may have more than one target, including more than one type of ion channel. About eight types of G-protein-linked K\(^+\) channels have been recognized in excitable cells (atrium, hippocampus, skeletal muscle) and a few were reported in clonal rat anterior pituitary cells (GHS cells) (Codina et al., 1987a; Yatani et al., 1987), insulin secreting cells (Eddelstone et al., 1989; Ribalet et al., 1989) and kidney (Light et al., 1989). Despite the progress made on the modulatory action of hormones and transmitters on K\(^+\) channels, several major questions still remain. What is the molecular mechanism of channel modulation? Do G-proteins actually directly bind to the channel molecule(s) thus modulating them, or are intermediate membrane proteins involved?

Several neurotransmitters and second messengers can modify the activity and function of K\(^+\) channels in epithelial cells. Somatostatin (SST) inhibited secretion and reduced intracellular Ca\(^{2+}\) in GH3 cells resulting in a cAMP-independent hyperpolarization. Acetylcholine (ACh) has the same effect, suggesting that SST and ACh directly activate the same subset of GH\(_3\) K\(^+\) channels (Codina et al., 1987a; Yatani et al., 1987). In the cases thus far known, the channel is a separate molecule from the receptor, and the effects on the channel are mediated by second messengers activated as a result of neurotransmitter-receptor interaction. Co-localization of the receptor and channel protein molecules in the same small area of the cell membrane may be an important factor in determining the response time (Toro and Stefani, 1991).

**POTASSIUM CHANNEL OPENERS**

One major goal of K\(^+\)-channel study is to discover chemical compounds that selectively open K\(^+\) channels, the so-called channel openers. Three compounds in this category are nicorandil (concentration causing 50% inhibition (IC\(_{50}\)), 30-300 \(\mu\)M), pinacidil (IC\(_{50}\) = 2.5-40 \(\mu\)M), and etomoxil (IC\(_{50}\) = 20-40 \(\mu\)M) (Cook and Quast, 1990; Robertson and Steinberg, 1990); they hyperpolarized the membrane by increasing K\(^+\) permeability through what appear to be two different mechanisms (Robertson and Steinberg, 1990), and they opened maxi Ca\(^{2+}/\)K\(^+\) channels incorporated into planar lipid bilayers (Gelband et al., 1989). Electrophysiological evidence indicated that the drugs can affect ATP-sensitive K\(^+\) channels in pancreatic \(\beta\)-cells (Cook and Quast, 1990), causing an effect similar to that seen in cardiac cells and vascular smooth muscles (Quast and Cook, 1989).

Diazoxide opens ATP-sensitive K\(^+\) channels in \(\beta\)-cells, leading to increased secretion of insulin (50% effective concentration (EC\(_{50}\)), 20-100 \(\mu\)M) (Cook and Quast, 1990; Robertson and Steinberg, 1990). The effect may be mediated by a second-messenger system that phosphorylates the channel (Petersen and Dunne, 1985), and it appears to be more potent than that due to other openers that open pancreatic ATP-sensitive K\(^+\) channels (Codina et al., 1987b). The effect of diazoxide on cell secretion may have therapeutic applications (Cook and Quast, 1990).

Do potassium channel openers activate K\(^+\) channels or do they inhibit channel inactivation? What is the nature of the antagonism of K\(^+\) openers by sulphphonylureas? These questions cannot presently be answered satisfactorily, and further investigations are needed.

K\(^+\) channel openers may have a pharmacological role in a variety of nonexcitable tissues. In asthma, openers may act via a second-messenger cascade in airway epi-thelial cells, activating Ca\(^{2+}/\)K\(^+\) channels, producing membrane hyperpolarization (McCann et al., 1990). In the urinary tract, the pharmacological effects of K\(^+\)-openers may reflect the Na\(^+/-\)K\(^+\) co-transport function, resulting in bladder hyperactivity (Wang et al., 1992). In pancreatic \(\beta\)-cells K\(^+\)-openers could open ATP-dependent K channels, leading to increased insulin secretion.

**POTASSIUM CHANNEL BLOCKERS**

Epithelial cells exhibit a variety of K\(^+\) channels having different gating properties; this variety compli-
cates the interpretation of \(K^+\)-channel currents and their modulations by drugs. Discovery of a number of toxins that block different \(K^+\) channels has facilitated study of channel diversity, but no blockers are available that specifically block one type of \(K^+\) channel completely without affecting other \(K^+\) channels or other classes of ion channels. The reviews that dealt with channel peptide blockers focused mainly on effects in excitable cell membranes (Castle et al., 1989; Cook and Quast, 1990; Dreyer, 1990; Strong, 1990).

Venom of the bee *Apis meliora* contains apamin, a single chain peptide consisting of 18 amino acids; it binds to \(Ca^{2+}/K^+\) channels of guinea pig liver (Cook et al., 1983), colon (Hughes et al., 1982), rabbit liver (Guggino et al., 1987), bovine adrenal cortex (Guggino et al., 1987) and hepatocytes (Capoid and Ogden, 1989; Romey et al., 1984). Apamin selectively inhibited ATP-induced hyperpolarization and decreased the activity of \(K^+\) channels in hepatocytes (EC\(_{50}\) = 20-100 \(\mu\)M, IC\(_{50}\) = 240 pM-10 nM) (Banks et al., 1979; Burgess et al., 1981; Cook and Quast, 1990; Dreyer, 1990; Strong, 1990). There are reports that apamin did not block \(Ca^{2+}/K^+\) channels in erythrocytes (Burgess et al., 1981) and in pancreatic \(\beta\)-cells (Lebrun et al., 1983).

Apamin is selective for \(Ca^{2+}/K^+\) voltage-dependent channels with small conductance. It is not certain whether it acts by changing the gating properties of the channel (reduction of the open time, decreasing the probability of the channel to be in open state) or by blocking the channel conductance. In an experiment on \(Ca^2+/K^+\) channels in pancreatic \(\beta\)-cells, apamin induced burst activity of the channel by an unknown mechanism (Ammala et al., 1993; Rosario, 1985). Mast-cell degranulating peptide had a small effect on \(K^+\) channels in guinea pig hepatocytes (Hughes et al., 1982), but at 10 times the concentration of apamin.

\(ChTx\) acts as a specific blocker of \(Ca^{2+}/K^+\) channels (EC\(_{50}\) = 2 nM; IC\(_{50}\) = 1-30 \(\mu\)M) (McCann et al., 1990; McCann and Welsh, 1990); it is a single-chain polypeptide with 37 amino acids (Dreyer, 1990; Gimenez-Gallego et al., 1988; Valdivia et al., 1988). Probably, its action is to bind to the external mouth of the channel (McCann et al., 1990). The idea of physical occlusion of the ion channel is further supported by experiments using both TEA and \(ChTx\) blocks; the results demonstrated that when TEA blocked \(K^+\) channels from outside, \(ChTx\) could not attain its binding site. The blocking effect of \(ChTx\) is reversible (Cook and Quast, 1990).

\(Ca^{2+}/K^+\) channels in \(GH_3\) cells (Gimenez-Gallego et al., 1988) were used to monitor the biological activity of \(ChTx\) during its purification. At 2 nM, the low-conductance \(Ca^{2+}/K^+\) channels were affected, but not the delayed outward \(K^+\) channels nor the other types of ion channels (\(Ca^{2+}\) and \(Na^+\)). Two types of voltage-activated \(Ca^{2+}\)-insensitive \(K^+\) channels in T-lymphocytes (18 pS) were found to be completely blocked by 5 nM \(ChTx\) (Lewis and Cahalan, 1988; Wolff et al., 1988).

Other useful peptide toxins include \(\beta\)-bungarotoxin, crotoxin, notexin, taipoxin and dendrotoxin, which are derived from snake venoms (Cook and Quast, 1990; Quast and Cook, 1989). They affect \(Ca^{2+}/K^+\) channels in neuronal membranes, but there are no reports on their action on \(K^+\) channels in epithelial cell membranes. It has been suggested (Dreyer, 1990; Strong, 1990) that some of them may have a slight effect on secretion in hepatocytes, pancreatic \(\beta\)-cells and renal cells; in these reports, inside-out or outside-out patches (20 pS) were studied, and the toxins reduced the unitary conductance in the presence of a physiological \(K^+\) gradient.

**BIOPHYSICAL MECHANISMS**

The basic model of the \(K^+\) channel consists of a mouth (an area of nonrestricted diffusion), a tunnel (a place where the applied voltage is dropped), and a selective filter within the tunnel (Latorre and Miller, 1983). From experiments with channel blockers and openers it is necessary to postulate the existence of a large mouth which contains hydrophobic regions; this probably involves the hydrocarbon region of the lipid bilayer, which prevents cations from entering. The conductance of \(K^+\) channels, however, appears to exceed the limits imposed by the rates at which ions diffuse through aqueous solutions. It is difficult to model the kinetics of a transport mechanism that can be faster than unrestricted diffusion, and yet give high ion selectivity.

The first-order processes whereby the ion enters or leaves the channel structure must be clearly separated (Latorre and Miller, 1983). One way to explain the exit rate is to hypothesize that the channel can accumulate more than one ion. In this model, one ion lessens the electrostatic forces on the second; in effect, the second ion pushes the first one out of the channel. This mechanism tends to explain an increased level of conductance, but it is not possible to calculate the magnitude of the effect because the channel structure is not well known.

Increased conductance could be explained in terms of the length of the restricted diffusion pathway. By assuming that the channel's length is less than several nanometers, the maximum channel conductance can...
be brought into an acceptable range. Another solution is to assume that the channel's radius is increased, enlarging the area available for capture by the channel; a wide channel mouth would lead to a large channel conductance (Jan and Jan, 1992; Latorre and Miller, 1983). These proposed mechanisms could only partially explain the high channel conductance. The full explanation probably involves the region where the ion channel selectivity mechanism is located, the so-called ion-channel selective filter.

Some K⁺ channels exhibit bursting behavior without the addition of chemicals to either side of the membrane (Geletyuk and Kazachenko, 1989). This burst function and its frequently associated multilevel channel conductance are voltage- and concentration-dependent, and show a tendency for cluster formation (Liebovitch et al., 1987). Bursting activity may be due to fluctuations in effective pore diameter when the ion passes through the channel. The occurrence of multilevel conductance suggests that the channel is organized into a number of synchronized subunits, leading to the occurrence of fluctuations in channel conductance. Alternatively, channel bursting function may be due to the transitions between different conformational modes of the channel.

Single-channel experiments and modeling have shown that partial dehydration of the permeant ions must occur at the selective filter by means of an exchange with ligand groups of the channel protein (Latone and Miller, 1983; Yellen, 1987). The filters of maxi Ca²⁺/K⁺ channels with high selectivity and inward rectifiers precisely fit the K⁺ ion; in other types of K⁺ channels, the structure is wider and allows some movement during which the ion cannot interact with the filter.

A K⁺ channel gene has been cloned and sequenced (Baumann et al., 1988; Kamb et al., 1988; Papazian et al., 1987; Tempel et al., 1988; Timpe et al., 1988), expressed in Xenopus oocytes (Isacoff et al., 1990; Iverson et al., 1988; Kubo et al., 1993b; Ruppersberg et al., 1990; Stühmer et al., 1988; Timpe et al., 1988), and reconstituted in planar lipid bilayers (Constantin et al., 1990). The protein contained six membrane-spanning segments, and exhibited an architecture and amino acid sequences similar to those of the voltage-dependent sodium channels (Noda et al., 1984).

The Shaker K⁺ channels vary in their amino-acid structure (Jan and Jan, 1990, 1992), leading to drastic effects on kinetic properties during expression in Xenopus oocytes (Hoshi et al., 1990; Iverson et al., 1988; Timpe et al., 1988). These structural changes appear to generate the diversity of K⁺ channels (Pongs, 1989). K⁺ channels in different cells may be composed of a number of different subunits (Hoshi et al., 1990; McCormack et al., 1990). It has not been determined whether all K⁺ channels are related to the currently predicted structure of the Shaker K⁺ channel family.

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