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Topical Review

Ion Channel Subconductance States

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Introduction

The simplest model for an ion channel has only two states: open (and so, conducting) and closed (nonconducting). Although alamethicin, excitability inducing material, and hemocyanin channels in bilayers showed multiple conductance levels (for a review, *see* Latorre & Alvarez, 1981), early descriptions of biological channels based upon fluctuation analysis were compatible with the simple two-state model (Conti et al., 1976; Neher & Stevens, 1977; Sigworth, 1977), as were initial patch-clamp results (Neher & Sakmann, 1976; Horn & Patlak, 1980; Sigworth & Neher, 1980; Horn, Patlak & Stevens, 1981). However, single-channel measurements using both the patch-clamp techniques and the technique of incorporating channel proteins into artificial membranes have since shown that many types of ion channels do not conform to a simple two-state model, but instead exhibit more than one open-state conductance. The less frequently occurring conductance levels are often referred to as substates. Most frequently, substates have lower conductance than the main-state, although this is not always so. Examples of single-channel records that show ion channel substates are shown in the *figure*.

The study of subconductance states may aid our understanding of ion channel structure and physiology and help us to better understand ion permeation mechanisms. Subconductance states may provide us with information about channel structure by, for example, indicating possible subunit structure of the channel molecules, or cooperativity between channels. The appearance of conductance substates following channel modification, as for the gramicidin channel (Busath & Waldbillig, 1983) provides information about specific channel amino acid residues which can alter ion permeation. Although in most cases substates are observed only rarely, regulation of their occurrence may have physiological significance (Hamill, Bormann & Sakmann, 1983).

The Criteria for Recognizing Substates

In order to investigate subconductance states, one must be able to differentiate between substates of a single population of channels and separate channel populations. Substate activity can be identified by the following characteristics. First, a channel substate should interconvert with the channel main-state; thus, direct transitions from one conductance level to another should be observed. Second, the substate should only be observed in the presence of channel main-state activity. Common observation of the putative channel substate in the absence of the main-state is a good indication that one is observing separate channel populations, and not multiple open states of the same channel. In many cases the substate comprises only a small fraction of the channel open time, so that the converse need not be true. Third, one must exclude the possibility that the main-state is the superposition of two independent channels. For example, to determine whether a 100 pS channel has a 60 pS substate, 60 pS and 100 pS transitions, but no transitions to 40 pS, must be observed.

Channel subconductance states commonly have the same ionic selectivity and response to pharmacological agents as the main-state (*see below*). Such similarities in channel properties have been used as evidence that the substate is indeed due to the same channel as the main-state. However, this is not always the case. Differences in ion selectivity between main-state and substate have

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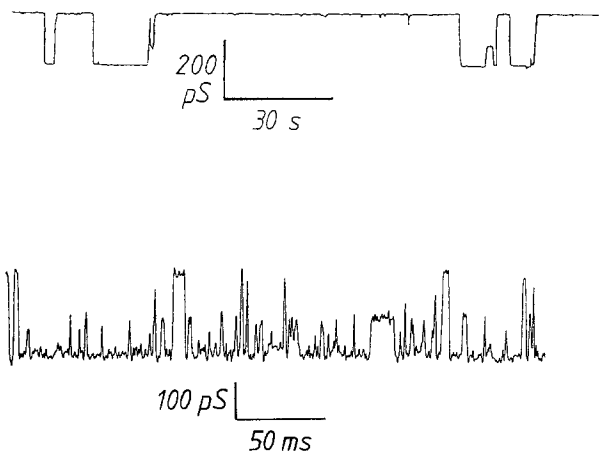

Fig. Channel openings downward in both traces. Top: Single-channel current record showing main and substate conductance of the rabbit SR potassium channel. These channels were incorporated into an artificial membrane containing phosphatidylethanolamine, phosphatidylserine, cholesterol and decane. 40 mV were applied. The bathing solution was 0.1 M K_2SO_4. Bottom: Current record from a nonselective channel from human retinal pigment epithelial cell in culture. Inside-out patch in symmetric 0.075 M KCl. 20 mV were applied

been found for some channels (Szabo & Busath, 1982; Busath & Szabo, 1984; Hanke, 1984). Different responses to blockers have been found for the open states of the potassium-selective channel of frog sarcoplasmic reticulum (Labarca & Miller, 1981) and the acetylcholine receptor channel (Takeda & Trautmann, 1984).

It is possible to confuse an altered kinetic state with a subconductance state due to bandwidth limitations in the recording apparatus. This problem could arise if a channel flickered rapidly in the new state. Flickering at a frequency beyond the bandwidth of the recording amplifier would lead to an apparent decrease in the open channel conductance (Yellen, 1984), with the apparent conductance being the time average of the channel current (Läuger, 1985). Flicker frequencies greater than, but near to, the cut-off of the amplifier, would produce increased noise in the apparent substate (Läuger, 1985). Flickering of a channel could not produce apparent substates of greater conductance, nor could a single kinetic state produce apparent multiple subconductances.

Experimental manipulations which might alter the flicker rate could be used to help determine whether decreased conductance states were due to changes in channel conductance or kinetics. Changes in the flicker rate might alter both the apparent conductance of the substate and the shape of channel amplitude histograms (Yellen, 1984). Thus, changes in the ratio of main-state to substate conductance under conditions known to affect channel kinetics might be an indication of kinetic, and not conductance, substates.

Lowered temperature has been reported to enhance the probability of observing some subconductances (Hamill et al., 1983; Gustin et al., 1986). Channel kinetics are affected by temperature (e.g., Labarca, Coronado & Miller, 1980). Although ion channel conductance and selectivity are also functions of temperature, their temperature coefficients are low (Hille, 1975). Thus, the ratio of substate to main-state conductance might be expected to change little at different temperatures for a true subconductance state, while this ratio might be much more temperature dependent for an apparent substate due to channel flickering. Measurements at several temperatures might then help to differentiate between kinetic and conductance substates.

Ion channel kinetics are often affected by membrane lipid composition (Latorre & Donovan, 1980; Latorre & Alvarez, 1981; Boheim et al., 1982). Incorporation of channels into membranes of different lipid compositions may also help to differentiate between kinetic and conductance substates. However, alteration of membrane lipids may also affect channel conductance (Rosenberg & Finkelstein, 1978; Latorre & Alvarez, 1981; Pope, Urban & Haydon, 1982).

Substates Described in the Literature

Acetylcholine Receptor Channel Substates

Patch-clamp recordings from cultured embryonic rat muscle membranes have demonstrated a substate of the acetylcholine receptor (AChR) channel with a conductance 40% that of the most frequent conductance state (Hamill & Sakmann, 1981; Trautmann, 1982; Takeda & Trautmann, 1984). (AChR channels in embryonic muscle usually have conductances of about 35 pS, but occasionally mature 50-pS channels are also observed. Both large conductance states have the same lower conductance substate (Hamill & Sakmann, 1981).) The ionic selectivity of the substate is similar to that of the mainstate. When Hamill and Sakmann (1981) replaced sodium with either lithium or cesium, both the main-state and substate conductance changed proportionately. Fast, flickery closures to a very low conductance substate of the nicotinic AChR channel have also been described (Auerbach & Sachs, 1982, 1984; Sachs, 1983). Current carried by the channel in this substate is proportional to the main-state current and appears to have a reversal

potential similar to that of the main-state (Auerbach & Sachs, 1982).

Transitions directly from one open state to another indicate that the AChR conductance levels are due to the same channel molecules and do not arise from separate channel populations. Although Hamill and Sakmann (1981) never observed an opening to a substate preceding an opening to a main conductance state, Trautmann (1982) observed transitions to the substate which preceded as well as those which followed main-state openings. Auerbach and Sachs (1984) found no evidence that either the main-state or the substate were far from kinetic equilibrium. Takeda and Trautmann (1984) observed nearly the same number of substates preceding the main-state as following it. Openings to the substate also occur independently of openings to the main-state (Hamill & Sakmann, 1981; Trautmann, 1982; Takeda & Trautmann, 1984).

A different kind of subconductance behavior has been observed with AChR channels from *Torpedo* electroplax incorporated into artificial bilayers. Schindler, Spillecke and Neumann (1984) describe experiments in which either purified AChR channel monomers or purified dimers were incorporated into bilayers. The results show that both monomer and dimer forms of the AChR molecule may act as conducting units, with the dimer having twice the conductance of the monomer. The dimer conductance most closely approximates that for AChR channels in vivo, suggesting that in vivo both subunits of the dimer tend to act in unison. Both the monomer and dimer channels show a substate of lower conductance.

Potassium Channel Substates

The inward rectifier channel from guinea-pig ventricular cells has several conductance substates (Sakmann & Trube, 1984). The most frequently observed substate has a conductance approximately three-fourths that of the main conductance. Direct transitions between the main-state and the substate were observed, with the substate sometimes preceding and sometimes following the main-state. Openings to the substate independent of main-state openings were also observed. In addition, rare events were observed with current levels one-fourth, one-half, and five-fourths of the main-state. The current-voltage relations for all conductance states of this channel extrapolated to the same reversal potential.

Potassium-selective channels from the sarcoplasmic reticulum (SR) of frog skeletal muscle (Labarca & Miller, 1981), mammalian heart muscle (Tomlins, Williams & Montgomery, 1984; Hill, Coronado & Strauss, 1986), and skeletal muscle (Fox, 1985; Tomlins & Williams, 1986) exhibit conductance substates. In frog, both open states are commonly observed, have conductances in 0.1 M KCl of 50 and 150 pS, and have identical ionic selectivities (Labarca & Miller, 1981). Openings to the 150 pS state are always preceded by openings to the 50 pS state. Cesium blocks current through both open states in a voltage-dependent manner, although the voltage dependence differs for the two states. The cesium blocking site senses a larger fraction of the transmembrane potential when the channel is in the 150 pS state than when the channel is in the 50 pS state (Labarca & Miller, 1981).

The potassium-selective channels from the SR of mammalian heart and skeletal muscle differ somewhat from those of frog skeletal muscle. The lower conductance state occurs much less frequently than does the larger conductance state and does not necessarily precede main-state openings (Fox, 1985). Its conductance is approximately 60% of the main-state (Tomlins et al., 1984; Fox, 1985; Hill et al., 1986; Tomlins & Williams, 1986). More current noise is observed when the channel is in the substate than in the main-state (Tomlins et al., 1984; Fox, 1985; Tomlins & Williams, 1986) and the conductance is more variable (Tomlins & Williams, 1986). The dissociation constants for both potassium and thallous ion are virtually identical for both channel open states (Fox, 1985). Tomlins et al. (1984) and Tomlins and Williams (1986) also find that the ionic selectivities of both states are very similar. Decamthonium has similar blocking effects on both conductance states (Tomlins et al., 1984). Small amounts of thallous ion in the presence of potassium greatly decreases the conductance of these channels (Fox & Ciani, 1985). The ratio of substate to main-state conductance is the same at all mole fractions of thallous ion in these mixed salt solutions (Fox, 1985).

A patch-clamp study of rabbit smooth muscle cells described potassium channels with sublevels of conductance of about two-thirds and, more rarely, one-third the main-state conductance (Benham & Bolton, 1983). Most transitions of this channel were between closed and fully open, but direct transitions between fully and two-thirds open were observed, as well as transitions between the sublevels. The channel substates had the same reversal potential as the main-state.

A potassium-selective channel from HeLa cells has been described (Sauvé, Roy & Payet, 1983) that has a main-state conductance of 40 pS and a substate conductance of 20 to 30 pS in 0.15 M KCl. The same reversal potential was found for both conductance states.

Two open states with similar reversal potentials were also observed in a potassium channel found in yeast (Gustin et al., 1986). This channel had a substate of higher conductance, which was observed at 5°C. The ratio of substate to main-state conductance appeared to be independent of the ionic composition of the bathing medium.

A voltage-dependent potassium-selective channel from *Torpedo* electroplax has been observed in artificial membranes that has two open-state conductances. In 0.2 M potassium salt the conductance levels of this channel are 70 and 150 pS; in 0.2 M sodium salt the conductances are 12 and 30 pS (Hanke, 1984). Thus, in this channel, the ratio of the conductances depends on the ionic composition of the bathing solution.

The calcium-dependent potassium channel of cultured rat muscle (Barrett, Magleby & Pallotta, 1982) and the serotonin-dependent potassium channel from *Aplysia* sensory neurons (Siegelbaum, Camardo & Kandel, 1982) also exhibit substate conductances.

Substates Observed in Other Cation Channels

The single-channel conductance of a glutamate-activated cation channel observed in cultured rat hippocampal neurones appears to depend on the agonist that activates the channel (Jahr & Stevens, 1986). Single channels activated by glutamate ranged from 5 to 60 pS in conductance. Transitions were observed between practically all levels. The glutamate analog NMDA mainly activated channels with conductances of 40 to 60 pS, while quisqualate and kainic acid mainly activated single channels with conductances below 20 pS. The reversal potentials of the channels with conductances lower than 25 pS were unaffected by calcium ion concentration changes, although these changes affected the reversal potentials of the larger conductance levels.

Most studies of sodium channel currents show no evidence of subconductance states (Sigworth & Neher, 1980; Horn et al., 1981; Kreuger, Worly & French, 1983). In some cases (Nagy, Kiss & Hof, 1983; Kunze et al., 1985) multiple sodium channel conductances have been described. However, it is not clear whether these findings indicate separate channel populations or subconductance states. I am not aware of published examples of calcium channel subconductance states.

Anion Channel Substates

Subconductance states have also been described in anion channels. Hamill et al. (1983) observed substate conductances of chloride channels from spinal cord neurons in culture. They observed two conductance levels for gamma-amino butyric acid (GABA)-activated chloride channels, and three levels for glycine-activated chloride channels in these cells. The most commonly observed conductance for the glycine-activated channels was 45 pS, while for the GABA-activated channels it was 30 pS. The larger substate of the glycine-activated channels also had a conductance of 30 pS, while both glycine- and GABA-activated channels had substates of 20 pS conductance. Both channels had the same selectivities, as determined by replacing chloride with various halides. These authors suggest that both GABA and glycine receptors might activate the same anion channel, with the main conductance state of the channel being determined by the receptor that activates it. Barker and McBurney (1979) had previously made a similar suggestion on the basis of fluctuation analysis of GABA- and glycine-activated currents in mouse spinal neurons.

Another type of substate is illustrated by the behavior of a chloride channel from *Torpedo* electroplax, which has been studied following incorporation into artificial bilayer membranes (Miller, 1982; Hanke & Miller, 1983). This channel exhibits periods of inactivity separating bursts of activity, with the open-channel conductance fluctuating between three states of 0, 10, and 20 pS in 0.2 M chloride salt. The larger open state has twice the conductance of the smaller under all ionic conditions examined. However, the 10-pS open state is never observed in isolation (Miller, 1982). This indicates that the slow gating process, which leads to long silent periods, must act on both open-state conductances as a unit. Thus, the observed conductance levels are not due to the activity of separate channels, but are due to the activity of a channel with multiple conductance states. This channel has been modeled as being composed of two identical subunits that are gated together, but which open and close independently when the gate is open (Miller 1982; Hanke & Miller, 1983).

A large-conductance anion channel has been found in B lymphocytes (Bosma, 1986). Besides the most common conductance of 400 pS in 0.165 M NaCl, the channel has five other conductance levels, at 10, 30, 50, 75, and 110% of the main-state level. In inside-out patches, the probability of observing a subconductance state decreases with time after seal formation. The reversal potential in asymmetric ion solutions for all anions tested is the same for all conductance states of the channel. The ratio of substate conductance to main-state conductance for all the substates is the same at three different concentrations of NaCl. Although chloride and

thiocyanate are nearly equally permeant, in mixtures of 10% thiocyanate-90% chloride, channel conductance is only about 70% that found in similar concentrations of each ion alone. The substate conductances are found in the same ratios in the mixed thiocyancate-chloride solution as in single-salt solutions. Thus the conductance properties of the substates seem to be identical, except in conductance magnitude, to those of the main-state.

Similar large-conductance (300–500 pS) anion-selective channels which have several substates have been described in rat muscle (Blatz & Magleby, 1983), rat Schwann cells (Gray, Bevan & Ritchie, 1984), apical membranes of A6 cells (Nelson, Tang & Palmer, 1984), mouse macrophages and chicken myotubes (Schwarze & Kolb, 1984), rabbit urinary bladder epithelium (Hanrahan, Alles & Lewis, 1985), apical membrane of MDCK cells (Kolb, Brown & Murer, 1985), rat lung alveolar epithelium (Schneider et al., 1985; Krouse, Schneider & Gage, 1986) and rat peritoneal mast cells (Lindau & Fernandez, 1986). The main-state conductance of these channels often appears to be an integer multiple of the lowest substate conductance (Gray et al., 1984; Nelson et al., 1984; Krouse et al., 1986). The substates of the large channel from rabbit urinary bladder showed the same reversal potentials as the main-state (Hanrahan et al., 1985).

These large-conductance anion channels resemble, in many respects, the VDAC channel observed in artificial membranes by Schein, Colombini and Finkelstein (1976). Mitochondrial VDAC channels from *N. crassa* are reported to insert into artificial membranes not singly, but as channel aggregates, most commonly as aggregates of three (Manella, Colombini & Frank, 1983). This behavior is similar to that of the channel-forming matrix protein from *Escherichia coli,* also called porin. The smallest conductance increases induced by these channels in artificial membranes characteristically decline in three equal conductance steps after their initial appearance in the membrane (Schindler & Rosenbusch, 1978). Electron microscopy indicates that three channels at the extracellular membrane face merge into one channel at the cytoplasmic face, with the area of the pore at the cytoplasmic face less than the sum of the cross-sectional area of the three pores at the other face (Engel et al., 1985). Careful measurements reveal that the first conductance step is actually 7% smaller in conductance than the second and third steps (Engel et al., 1985). This might be consistent with the disparity in pore areas if the cross-sectional areas of the individual pores are conductance-limiting when only two or one are open (Engel et al., 1985).

A calcium- and potassium-activated chloride channel from molluscan neurons has a conductance of 200 pS, which appears to be the sum of 16 identical substates of 12.5 pS (Geletyuk & Kazachenko, 1985). Unlike the channel found in B lymphocytes, the likelihood of observing subconductance states appears to increase with time after formation of the seal, as if the channel were formed of subunits that irreversibly close or degrade with time (Geletyuk & Kazachenko, 1985).

Substates Observed in Nonselective Channels

Large nonselective channels with conductances of 200 to 400 pS and several subconductance states have been reported for lens and corneal epithelium (Rae & Levis, 1984*a*, *b*; Rae, 1985), isolated rods (Sather, Bodoia & Detwiler, 1985) and retinal pigment epithelium (Fox et al., *submitted*[1]).

Purified bovine lens gap junction channel protein has been added to solutions bathing planar bilayers, leading to the appearance of large-conductance (200 pS in 0.1 M salt, 1500 to 2000 pS in 1 M salt), nonselective single-channel currents which have four equal subconductance states (Zampighi, Hall & Kreman, 1985). It is interesting to note that bovine lens junction protein is composed of four identical subunits (Zampighi et al., 1982).

Possible Mechanisms of Subconductance Behavior

Two general types of subconductance state behavior have been observed. The first type is often called a "subunit" type, and the second, a "partial closure" or "partial opening" substate.

Subunit-type behavior occurs when channel conductance changes in increments of equal conductance, as if one, then two or several identical subunits are being recruited. The opening or closing of identical subunits would change channel conductance, but not other channel properties. The chloride channel from *Torpedo* electroplax in artificial bilayers shows this type of behavior. Similar behavior is seen with channels formed in artificial bilayers following incorporation of mitochondrial or bacterial proteins (Schindler & Rosenbusch, 1978; Manella et al., 1983; Engel et al., 1985), gap junction protein (Zampighi et al., 1985) and with anion channels and nonselective channels found in many tissues (*see above*). The ability of AChR channel monomers in bilayers to combine to form channels

[1] Fox, J., Pfeffer, B., Fain, G. Single-channel recording from cultured human retinal pigment epithelial cells. (*submitted*)

that appear to act as a unit, and which are identical to those formed by AChR dimers (Schindler et al., 1984) demonstrates that this model is physically possible. In a channel of this type, one would expect the conductance and ionic selectivity properties to be the same for all substates. This is found to be the case for the *Torpedo* electroplax chloride channel (Miller, 1982; Hanke & Miller, 1983), the ubiquitous anion channel (Kolb et al., 1985; Bosma, 1986), and the calcium- and potassium-activated chloride channel (Geletyuk & Kazachenko, 1985). The porin channel (Engel et al., 1985) exhibits a form of subunit-type behavior, where conductance triplets are observed. In this case, the conductance substates can be related to the physical makeup of the channel protein.

The alamethicin channel has been modeled as a complex of alamethicin molecules, which together form a pore across lipid bilayers (Baumann & Mueller, 1974; Boheim, 1974). In these models, individual alamethicin molecules orient perpendicularly to the plane of the bilayer, aggregating like the staves of a barrel to form the walls of a pore. The observed steplike (but unequal) changes in single-channel conductance result from the addition or removal of alamethicin molecules from the pore complex. Thus, channels formed by subunit aggregation need not necessarily have equal substate conductances.

The second type of subconductance state behavior often appears as a partial closure of the open channel, although the substate may also occur alone, or before a full opening of the channel. In this type of substate behavior, a channel spends most of its open time in the larger (or largest) conductance state, and a minority of its open time in a state of lower conductance. The ionic selectivity of the partial state is usually the same as that of the main-state. Examples of this type of substate behavior are seen with AChR channels (Hamill & Sakmann, 1981; Trautmann, 1982; Takeda & Trautmann, 1984), potassium channels from HeLa cells (Sauvé, 1983), potassium channels from SR (Tomlins et al., 1984; Fox, 1985; Hill et al., 1986; Tomlins & Williams, 1986), and chloride channels from spinal neurons (Hamill et al., 1983).

Intermediate between these two classifications are the substates of the inward rectifier (Sakmann & Trube, 1984) and the potassium channels from rabbit smooth muscle cells (Benham & Bolton, 1983). In these examples, the most commonly observed substates appeared to be of the "partial closure" type, but, less frequently, smaller fractional states were observed which suggest that these channels might be composed of subunits with one quarter (Sakmann & Trube, 1984) or one third (Benham & Bolton, 1983) the conductance of the channel main-state.

Additionally, some subconductance states have larger conductances than the main-state. Such substates have been observed in heart inward rectifier channels (Sakmann & Trube, 1984), a yeast potassium channel (Gustin et al., 1986) and an anion channel from B lymphocytes (Bosma, 1986).

A conformational change of the channel protein that hindered access to, or exit from, the pore might lead to partial closure-type substates. Takeda and Trautmann (1984) found that curare increases the open time of the AChR channel in the main-state, but not the partially open state. One interpretation of this result is that a blocking site is inaccessible to curare when the channel is in the partially open state. Partial occlusion of the pore has also been suggested to explain rare, low conductance states of the gramicidin channel (Busath & Szabo, 1981).

An ion channel can be thought of as an enzyme that catalyzes ion translocation across a membrane (Hille, 1975; Latorre & Miller, 1983). Maximum channel conductance (G_{max}) at high ion concentration corresponds to the maximum rate of ion translocation through the pore, and the ion concentration at which the channel conductance is half maximal corresponds to the dissociation constant (K_m). Channel conformational changes that alter the K_m, the G_{max}, or both, could account for subconductance states over a wide range of ion concentrations.

In an Eyring model of a channel, ion entrance and exit rates are represented by jump rates over energy barriers (Hille, 1975; Läuger, 1985). In a simple two-barrier, one-site Eyring model, multiplying or dividing both entrance and exit rates by the same factor would change the maximum channel conductance by that same factor, since this term is proportional to the product of these rates divided by their sum (Läuger, 1985). However, the ion binding constant, being the ratio of the entrance to exit rates, would remain unchanged by this transition. Thus, a change that raised peak energies would leave ion binding unchanged, yet reduce G_{max}. The substate of the potassium channel from rabbit SR has a lower G_{max} than the main-state, but the same K_m (Fox, 1985). The selectivity sequence (for various ion species) would remain unchanged when the shift in peak energy was the same for all ions. Changes in image forces (Labarca & Miller, 1981) or membrane surface dipole potentials (Krasne, 1978) could account for such constant energy shifts.

Many channels, including the SR potassium channel and the calcium-activated potassium channel (which exhibit partial substates), are thought to have wide mouths leading to narrow selectivity filters (Latorre & Miller, 1983). Dani (1986) finds that ion binding to sites in a wide channel mouth will affect the apparent values of both G_{max} and K_m.

Such ion binding might account for substates with altered selectivity as well as conductance, such as are found in the *Torpedo* electroplax potassium channel (Hanke, 1984).

Experimental Manipulation Can Enhance the Probability of Observing Substates

Substates of the gramicidin channel are observed with altered current-voltage relations and selectivity (Busath & Szabo, 1981, 1984; Szabo & Busath, 1982). Busath and Waldbillig (1983) have found that ultraviolet irradiation of membranes containing gramicidin results in the appearance of low conductance substates of the gramicidin channel. The action spectrum for this effect peaks at 280 nm, corresponding to the absorbance maximum for gramicidin tryptophan residues. Thus these experiments pinpoint amino acid residues which are capable of modulating currents through the gramicidin channel.

Other experimental manipulations have also been used that make it easier to study channel substates. Low temperature (Hamill & Sakmann, 1981) and curare (Trautmann, 1982; Takeda & Trautmann, 1984) enhance the probability of observing the AChR channel substate. A potassium channel substate with increased conductance is observed in yeast at low temperature as well (Gustin et al., 1986). Membrane lipid composition may affect the likelihood of observing a channel substate. Heterogeneity in gramicidin channel conductance states increases with increasing cholesterol concentration in artificial bilayers containing the channels (Rosenberg & Finkelstein, 1978; Pope et al., 1982). Membrane surface charge, and the ionic composition of the bathing medium may also affect channel state (Schindler et al., 1984).

Conclusions

The variety and number of ion channels that have subconductance states demonstrates the generality of the phenomenon. These substates may be thought of as being conductance modes of ion channels, in analogy to the kinetic modes of some channels (Hess, Lansman & Tsien, 1984). Regulation of these modes may be of functional significance to cells, as suggested by the hypothesis that the conductance state of a chloride channel is determined by the receptor, either GABA or glycine, that activates it (Hamill et al., 1983). Study of subconductance states may also provide useful information about channel structure and permeation mechanisms. Equal conductance substates may be indicative of multimeric structure of the channel protein, as suggested by the substates observed in lens gap junction channels (Zampighi et al., 1985) and porin channels (Engel et al., 1985). Study of substates has identified specific amino acid residues important in ion permeation through gramicidin channels (Busath & Waldbillig, 1983). Patterns in the transitions between conducting states may be useful in describing channel gating. For example, the finding that the low conductance state of the frog SR potassium channel always precedes the high conductance state limits possible kinetic schemes to those without direct pathways from closed to the high conductance state (Labarca & Miller, 1981). Thus better understanding of ion channel subconductance states improves our understanding of ion channel structure, function and regulation.

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Substates of glutamate-activated channels from cerebellar neurons have been observed by Cull-Candy and Usowicz (*Nature (London)* **325**:525–528, 1987). They conclude that these channels may adopt several conductance levels, with the predominant level depending on the receptor that activates the channel. The work of Jahr and Stevens concerning glutamate-activated channels from hippocampal neurons has been published in *Nature (London)* **325**:522–525, 1987. A nonselective channel from *Aplysia*, with a main-state conductance of 100 pS in 600 mM salt, has been studied by Chesnoy-Marchais and Evans (*J. Membrane Biol.* **93**:75–83, 1986). This channel often exhibits two or three substates, which have different ionic selectivities as determined by reversal potential measurements.