SELECTIVITY OF THE Ca^{2+}-ACTIVATED AND LIGHT-DEPENDENT K^+ CHANNELS FOR MONOVALENT CATIONS

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ABSTRACT The ionic selectivity of the Ca^{2+}-activated K^+ channel of *Aplysia* neurons and of the light-dependent K^+ channel of *Pecten* photoreceptors to metal and organic cations was studied. The selectivity sequence determined from reversal potential measurements is $Tl^+ \geq K^+ > Rb^+ > NH_4^+ > Cs^+ > Na^+$, Li^+ and is identical to the sequence determined previously for voltage-dependent K^+ channels in a variety of tissues. Our results suggest that some physical aspect of the K^+ channel is conserved in phyllogenetically different tissues and cells.

INTRODUCTION

The permeability of excitable cells to various ions and to small organic molecules has provided information about the movement of charged molecules through voltage-dependent Na^+ and K^+ channels in nerve and muscle cells (Hille, 1975; Campbell, 1976; Gay and Stanfield, 1978; Reuter and Stevens, 1980) and through the end-plate channel at the neuromuscular junction (Huang et al., 1978; Dwyer et al., 1980; Adams et al., 1980). These studies suggest that ions move through separate aqueous pores, which contain a region that confers selectivity upon the channel. Of the three types of channels that have been studied in detail, the voltage-dependent K^+ channel appears to be the most selective. It is a cationic selective channel which is capable of discriminating between small monovalent ions and is poorly permeable to all but the smallest organic molecules (Hille, 1973; Gay and Stanfield, 1978; Reuter and Stevens, 1980). The physical basis for such selectivity is not fully understood, but it has been suggested that there is a short, specialized region of the channel located near its external mouth which is so narrow that those ions that pass through the channel must be partially dehydrated (Armstrong, 1975; Hille, 1975). There are other currents, found in different excitable cells, which are likely to be caused by the movement of K^+ ions. Such K^+ currents are activated by synaptic transmitters (Hutter and Trautwein, 1956; Gerschenfeld, 1971), by increases in intracellular Ca^{2+} (Meech, 1978) or by changes in external energy, e.g. light, mechanical, etc. (Naitoh and Eckert, 1973; Gorman and McReynolds, 1978). It is of general interest to determine the selectivity sequence for K^+ currents that are not voltage dependent. We report now that the selectivity sequence for the Ca^{2+}-activated K^+ current of molluscan nerve cells and the light-dependent K^+ current of a molluscan photoreceptor are identical to the sequence for the voltage-dependent K^+ current.

METHODS

Experiments were done on pacemaker neurons R-15 and L-6 of the abdominal ganglion of *Aplysia californica* and on the distal hyperpolarizing photoreceptor cells of the mollusc *Pecten irradians* (scallop). Many of the experimental procedures have been described in detail (Cornwall and Gorman, 1979; Gorman and Hermann, 1979). The Ca^{2+}-activated K^+ current of the *Aplysia* neuron soma membrane was activated by iontophoretic injection of Ca^{2+} ions from an internal microelectrode which was located close to the inner surface of the membrane. A standard two-electrode voltage-clamp arrangement was used in the *Aplysia* neuron study and the Ca^{2+} ions were injected (200 nA for 10 s) in the voltage-clamp mode so that there was no net flow of current across the membrane during the injection. The exposed cells were maintained in a modified Ca^{2+}-free medium which contained (in mmol/liter) 500 NaCl, 10 KCl, 60 $MgCl_2$, 50 μM tetrodotoxin, and 10 Tris at pH 7.8 and at 15°C. A Ca^{2+}-free medium was used to eliminate Ca^{2+} influx during depolarization and, therefore, indirect activation of K^+ channels caused by the rise in intracellular Ca^{2+}. The light-dependent K^+ current of the distal photoreceptors was activated by brief (200 ms) flashes of white light that was intense enough to just saturate the receptor current. A single electrode voltage-clamp arrangement (Bader et al., 1979) was used in the photoreceptor studies because of the small size of the cells. The photoreceptors were maintained in a modified Na^+-free medium which contained (in mmol/liter) 434 choline Cl, 9 KCl, 9 $CaCl_2$, 50 $MgCl_2$, and 15 Tris at pH 7.8 and at 16°C. A Na^+-free medium was used to eliminate the sizable non-light-dependent conductance of the membrane to Na^+ ions in the absence of light. In the test medium all the KCl and some or all of the NaCl or choline Cl salts were replaced with a test salt. The

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following salts were tested: LiCl, NaCl, KCl, RbCl, CsCl, $TlNO_3$ or Tl acetate, NH_4Cl, methylamine HCl and hydrazine 2HCl. The Tl^+ solutions were made entirely from nitrate salts (*Aplysia* neuron studies) or from acetate salts (scallop photoreceptor studies) because TlCl is insoluble at the concentrations used. These solutions were tested in comparison with K^+ solutions made from similar salts. Changes in junction potential were minimal when switching from K^+- to Tl^+-containing solutions. In the *Aplysia* studies, neurons were exposed to the test media continuously for at least 15 min (the time needed to complete the measurements), whereas in the scallop studies photoreceptors were exposed to the test media for no longer than ~3 min.

RESULTS

The method used to determine permeability ratios (P_X/P_K) for monovalent cations and organic molecules (X) was similar to that used by Hille and his collegues (Dwyer et al. 1980; Adams et al. 1980) where reversal potentials of current responses were measured when the external solution contained only K^+ or X^+ as permeable cations. If $V_{r,K}$ is the reversal potential in the presence of K^+ and $V_{r,X}$, the reversal potential when X^+ is the only permeable cation in the external solution, then P_X/P_K may be obtained from a modified form of the Goldman-Hodgkin-Katz equation (Hille, 1975)

$$\frac{P_X}{P_K} = \frac{[K]_o}{[X]_o} \exp\left[\frac{F(V_{r,X} - V_{r,K})}{RT}\right]$$

where $[X]_o$ and $[K]_o$ are the external concentration of X^+ and K^+.

Fig. 1*A* illustrates the experimental determination of P_{Rb}/P_K for a R-15 cell immersed first in 50 mM Rb^+ ASW and subsequently in 50 mM K^+ ASW. The reversal potential for the Ca^{2+}-activated K^+ current ($I_{K,Ca}$) was determined in each solution by plotting the difference current (see figure legend) flowing 1–2 s after the end of a local Ca^{2+} injection. $V_{r,Rb}$ was −44 mV and $V_{r,K}$ was −35 mV. The shift in reversal potential for two cells was −9 mV and −10 mV and the mean value for P_{Rb}/P_K was 0.69. Fig. 1*B* shows a similar determination for a distal photoreceptor immersed first in 50 mM K^+ ASW and subsequently in 100 mM Rb^+ ASW. The reversal potential for the light-dependent current ($I_{K,L}$) was determined in each solution by plotting the difference current (see figure legend) flowing about 60 ms after the onset of a 200-ms light flash. $V_{r,Rb}$ was −46 mV and $V_{r,K}$ was −50.5. The mean value for P_{Rb}/P_K based on the mean shift in reversal potential for three cells was 0.71.

The permeability ratios for all the cations tested are summarized in the first two columns of data in Table I. The ions are arranged in order of increasing size. The results from the two types of cells are very similar and are discussed together. Clear reversal potentials were measured in external solutions containing K^+, Tl^+, Rb^+, and NH_4^+. These ions pass through the K^+ channel with relative ease and fall between 2.66 and 3.00 Å in diameter. The rest of the ions are no more than 3% as permeable as K^+. For most of these ions the values given represent the upper limits determined from estimated reversal potential measurements. A clear reversal potential for Cs^+ ions was determined in the *Aplysia* neuron studies, but not in the photoreceptor studies. The selectivity sequence for the passage of alkali metal cations through the K^+ channel is $K^+ > Rb^+ > Cs^+ > Na^+, Li^+$. This sequence corresponds most closely to sequence IV of Eisenman (Eisenman, 1963). In addition to the alkali metal cations, Tl^+ and NH_4 were the only other cations tested which were measureably permeable.

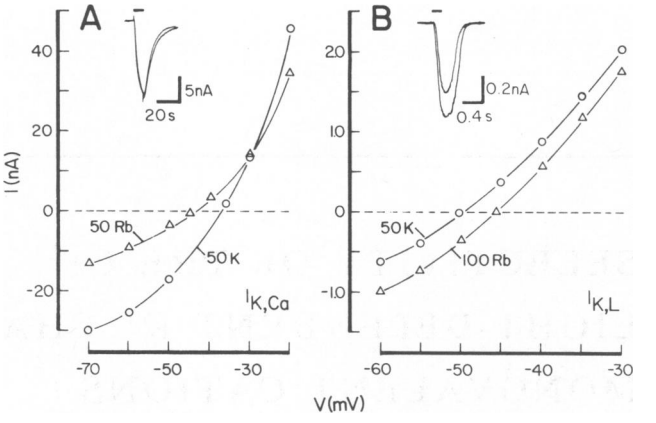

FIGURE 1 Current-voltage relations for the Ca^{2+}-activated K^+ current ($I_{K,Ca}$) and the light-dependent K^+ current ($I_{K,L}$) in Rb^+ and K^+-containing ASW. *A*, plot of the difference between the holding current and the peak current flowing 2 s after the end of a 10-s local internal Ca^{2+} injection in an *Aplysia* R-15 cell in an ASW solution containing 50 mM Rb^+ (Δ) and in a solution containing 50 mM K^+ (○) vs. membrane holding potential. *Inset*, records show comparable inward currents at −50 mV in 50 mM K^+ and at −90 mV in 50 mM Rb^+. The current in Rb^+ is slightly smaller, but is of similar time course. *B*, plot of the difference between the holding current the peak current flowing about 60 ms after the end of a 200-ms light flash in a distal, hyper-polarizing photoreceptor in an ASW solution containing 100 mM Rb^+ (Δ) and in a solution containing 50 mM K^+ (○) vs. membrane holding potential. *Inset*, records show inward currents at − 55 mV in 100 mM Rb^+ and −60 mV in 50 mM K^+. The current is somewhat smaller in K^+, but is of similar time course.

The reversal potential measurements would be in error if substantial changes in concentration occur on either side of the membrane during the period when K^+ channels are open. Such changes were minimized by making measurements using small currents near the reversal potential (see Fig. 1) to preclude large fluxes of K^+ or of the test ion. The reversal potential measurements might also be in error if any internal accumulation of the test ion occurred during the period of exposure (caused by movement of the test ion through leakage channels). This complication is more likely in the *Aplysia* neuron studies which took longer to complete than in the photoreceptor studies which were completed on a much shorter time scale. Measurements of the reversal potential in the presence of test ions were reasonably constant during the period of exposure in both cases, however, suggesting that any influx through leakage pathways is small.

TABLE I
PERMEABILITY RATIOS FOR CATIONS IN K^+ CHANNELS

Ion X	P_x/P_k					Minimum pore diameter
	$I_{k,Ca}$*	$I_{K,L}$†‡	$I_{K,v}$§	$I_{K,v}$‖	$I_{K,v}$¶	
						(Å)
Lithium	<0.011	<0.013	<0.018	0.09	<0.02	1.20
Sodium	<0.009	<0.008	<0.010	0.07	<0.03	1.90
Potassium	1.0	1.0	1.0	1.0	1.0	2.66
Thallium	0.99	1.07	2.3	1.29	—	2.80
Rubidium	0.69	0.71	0.91	0.74	0.95	2.96
Ammonium	0.11	0.11	0.13	0.15	—	3.00
Hydrazine	<0.04	<0.06	<0.029	—	—	3.30
Cesium	0.03	<0.023	<0.077	0.18	0.11	3.38
Methylamine	<0.04	<0.027	<0.021	—	—	3.60

*Ca^{2+}-activated K^+ current, *Aplysia* neurons.
‡Light-dependent K^+ current, scallop distal photoreceptors.
§Delayed K^+ current, myelinated axon (Hille, 1973).
‖Delayed K^+ current, *Helix* neurons (Reuter and Stevens, 1980).
¶Delayed K^+ current, frog skeletal muscle (Gay and Stanfield, 1978).

DISCUSSION

The results can be compared to measurements of permeability ratios determined for the delayed outward K^+ current (outward K^+ rectifier) of myelinated axon (Hille, 1973), of snail neurons (Reuter and Stevens, 1980) and of striated vertebrate muscle (Gay and Stanfield, 1978) (Table I). There is a remarkable similarity between the permeability ratios determined for voltage-dependent and non–voltage-dependent K^+ currents. The same ions pass through the two types of channels. Moreover, the selectivity sequence of Tl^+, $K^+ > Rb^+ > NH_4^+ > Cs^+ > Na^+$, Li^+ is preserved in different types of K^+ channels as well as in different cells and tissues. The Ca^{2+}-activated and light-induced K^+ channels appear to be a good deal less permeable to Tl^+ ions than the voltage-dependent K^+ channel in myelinated axon (Hille, 1973) and somewhat less permeable to Tl^+ than the voltage-dependent K^+ channels in snail neuron (Reuter and Stevens, 1980).

There is an additional similarity between the results from voltage-dependent and non–voltage-dependent K^+ channels. The addition of Cs^+ ions to an external K^+-containing medium blocks inward, but not outward K^+-current flow through voltage-dependent channels (Adelman and Senft, 1971; Hille, 1975; Hagiwara et al. 1976; Gay and Stanfield, 1977) presumably because Cs^+ can enter into but not pass through the channel. Inward Ca^{2+}-activated K^+ currents and inward light-dependent K^+ currents are also reduced appreciably after the addition of Cs^+ ions to external media containing K^+ ions.

The similarity between results suggest two general conclusions. First, the Ca^{2+}-activated and light-dependent K^+ channels are likely to be aqueous pores which contain a region with many of the same structural features found in the selectivity filter present in voltage-dependent K^+ channels. Second, it has been suggested that the selectivity filter and the gating machinery of voltage-dependent channels represent separate entities (Hille, 1978). Our results are consistent with this proposal and suggest that this separation is likely to be present in voltage- and in non–voltage-dependent channels. A logical inference from the results summarized in Table I is that some physical aspect of the K^+ channel is conserved in phyllogenetically different tissues and cells.

The construction of ionic channels in the plasma membrane during development is determined by genetic forces and may involve the assembly of modular units. It is possible that a selectivity filter is added to an aqueous pore of larger diameter during development so that the pore assumes the characteristics of the filter. The addition of different sensors which respond to an applied electric field, to a chemical transmitter, to an increase in intracellular Ca^{2+}, or to a change in light intensity (or to some other form of external energy) at the same or at a later stage in development would further define the operational characteristics of the channel.

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REFERENCES

Adams, D. J., T. M. Dwyer, and B. Hille. 1980. The permeability of endplate channels to monovalent and divalent metal cations. *J. Gen. Physiol.* 75:493–510.

Adelman, W. J., and J. P. Senft. 1958. Dynamic asymmetrics in the squid axon membrane. *J. Gen. Physiol.* 51:102s–114s.

Armstrong, C. M. 1975. Ionic pores, gates and gating currents. *Q. Rev. Biophys.* 1:179–210.

Bader, C. R., P. R. MacLeish, and E. A. Schwartz. 1979. A voltage-clamp study of the light response in solitary rods of the tiger salamander. *J. Physiol. (Lond.).* 296:1–26.

Campbell, D. T. 1976. Ionic selectivity of the sodium channel of frog skeletal muscle. *J. Gen. Physiol.* 67:295–307.

Cornwall, M. C., and A. L. F. Gorman. 1979. Contribution of calcium

and potassium permeability changes to the off response of scallop hyperpolarizing photoreceptors. *J. Physiol. (Lond.)*. 291:207–232.

Dwyer, T. M., D. J. Adams, and B. Hille. 1980. The permeability of the endplate channel to organic cations in frog muscle. *J. Gen. Physiol.* 75:469–492.

Eisenman, G. 1963. Cation-selective glass electrodes and their mode of operation. *Biophys. J.* 2(Suppl. 2):259–323.

Gay, L. A., and P. R. Stanfield. 1977. Cs^+ causes a voltage-dependent block of inward K^+ currents in resting skeletal muscle fibers. *Nature (Lond.)*. 267:169–170.

Gay, L. A., and P. R. Stanfield. 1978. The selectivity of the delayed potassium conductance of frog skeletal muscle fibers. *Pflügers Arch. Eur. J. Physiol.* 378:177–179.

Gerschenfeld, H. M. 1971. Serotonin: two different inhibitory actions on snail neurons. *Science (Wash., D.C.)* 171:1252–1254.

Gorman, A. L. F., and A. Hermann. 1979. Internal effects of divalent cations on potassium permeability in molluscan neurons. *J. Physiol. (Lond.)*. 296:393–410.

Gorman, A. L. F., and J. S. McReynolds. 1978. Ionic effects on the membrane potential of hyperpolarizing photoreceptors in the scallop retina. *J. Physiol. (Lond.)*. 275:345–355.

Hagiwara, S., S. Miyazki, and P. Rosenthal. 1976. Potassium current and the effects of cesium on this current during anomalous rectification of the egg cell membrane of a starfish. *J. Gen. Physiol.* 67:621–638.

Hille, B. 1973. Potassium channels in myelinated nerves. *J. Gen. Physiol.* 61:669–686.

Hille, B. 1975. Ionic selectivity of Na and K channels of nerve membrane. *In* Membranes, Lipid Bilayers and Biological Membranes. Dynamic Properties. Vol. 3. G. Eisenman, editor. Marcel Dekker, Inc., New York. 253–323.

Hille, B. 1978. Ionic channels in excitable membranes. *Biophys. J.* 22:283–294.

Huang, L. M., W. A. Catterall, and G. Ehrenstein. 1978. Selectivity of cations and nonelectrolytes for acetylcholine-activated channels in cultured muscle cells. *J. Gen. Physiol.* 71:397–410.

Hutter, O. F., and W. Trautwein. 1956. Vagal and sympathetic effects on the pacemaker fibres in the sinus venosus of the heart. *J. Gen. Physiol.* 39:715–733.

Meech, R. W. 1978. Calcium-dependent potassium activation in nervous tissues. *Annu. Rev. Biophys.* 7:1–18.

Naitoh, Y., and R. Eckert. 1973. Sensory mechanisms in *Paramecium*. *J. Exp. Biol.* 59:53–65.

Reuter, H., and C. F. Stevens. 1980. Ion conductance and ion selectivity of potassium channels in snail neurons. *J. Membr. Biol.* 57:103–118.