Membrane Permeability: Cation Selectivity Reversibly Altered by Salicylate

Abstract. *The effect of salicylate on the relative cation permeability of a membrane was investigated in large, identified molluscan neurons, with the use of intracellular recording techniques. Salicylate caused a reversible, dose-dependent decrease in the permeability of rubidium, cesium, sodium, and lithium ions relative to that of potassium ions. The results suggest that the changes in cation selectivity result from the adsorption of salicylate anions to the membrane with a subsequent increase in the density and field strength of anionic sites in the membrane.*

Salicylate increases the K^+ permeability and decreases the Cl^- permeability of human erythrocytes (*1*) and molluscan neurons (*2*). The permeability changes cause an increase in the membrane potential and input conductance of the molluscan neurons, which reduces the effect of synaptic input and the probability of generating an action potential output (*2*). The ability of salicylate derivatives to increase membrane potential and conductance is highly correlated with the octanol-water partition coefficient of these organic anions, and stereospecificity does not appear to be a requirement for drug activity (*3*). The results suggest that organic anions like salicylate change membrane permeability by adsorbing to membranes.

This report is concerned with the mechanism by which adsorption of salicylate anions to a membrane alters membrane permeability to inorganic ions. We have used Eisenman's theory of ion selectivity as the basis for our study (*4–6*). Eisenman has demonstrated that the principal factor controlling the discrimination of alkali cations (Na^+, K^+, Rb^+, Cs^+, and Li^+) by glass electrodes is the field strength of anionic sites in the glass (*4*). He suggested that the selectivity patterns found in different biological membranes are due to differences in the field strength of membrane anionic sites (*5, 6*). Considerations of his research have led us to propose that the adsorption of organic anions onto a biological membrane increases the density of negative sites and thus the anionic field strength, thereby altering cation and anion permeability. We have examined one aspect of this hypothesis by investigating the effect of salicylate on the alkali cation selectivity of neuronal membranes and comparing these results with predictions based on the Eisenman model.

Experiments were conducted on large, identified neurons in the buccal ganglion of the marine mollusk *Navanax inermis* (*7*). The ganglion was pinned to paraffin in a 2-ml plastic dish, and neurons were impaled with double-barreled micropipettes filled with 3*M* KCl; one barrel was used to record the membrane potential, and the other allowed passage of current across the membrane. We determined the relative permeability of the passive membrane to alkali cations by bathing the ganglion in a solution containing tris sulfate [tris (hydroxymethyl) aminomethane sulfate], magnesium sulfate, mannitol, and the sulfate salt of either Na^+, K^+, Cs^+, Rb^+, or Li^+ and monitoring the membrane potential as the species and concentration of the cation were changed (*8*).

The relative cation permeability was approximated by applying the analysis of Hagiwara *et al.* (*9*) to the Goldman-Hodgkin-Katz equation (*10*). In this analysis tris and sulfate are considered relatively impermeant substitutes for monovalent cations and anions, respectively, and the internal Na^+ and Cl^- concentrations are presumed to be negligible in the steady state (*9*). Under these conditions, when the membrane potential in cation species X^+ at external concentration $[X^+]_0$ equals that in K^+ at $[K^+]_0$, then (*9*)

$$P_X/P_K = [K^+]_0/[X^+]_0$$

where P_X is the membrane permeability to cation species X^+ and P_K is the K^+ permeability.

Examples of the variation in membrane potential with the concentration and species of the monovalent cation are illustrated in Fig. 1. In control conditions (Fig. 1A) the membrane potential decreased in a nonlinear fashion with the logarithm of the K^+, Rb^+, and Cs^+ concentrations and did not vary with changes in the concentration of Na^+ or Li^+ over the concentration range shown. The nonlinearity may be due to an increased

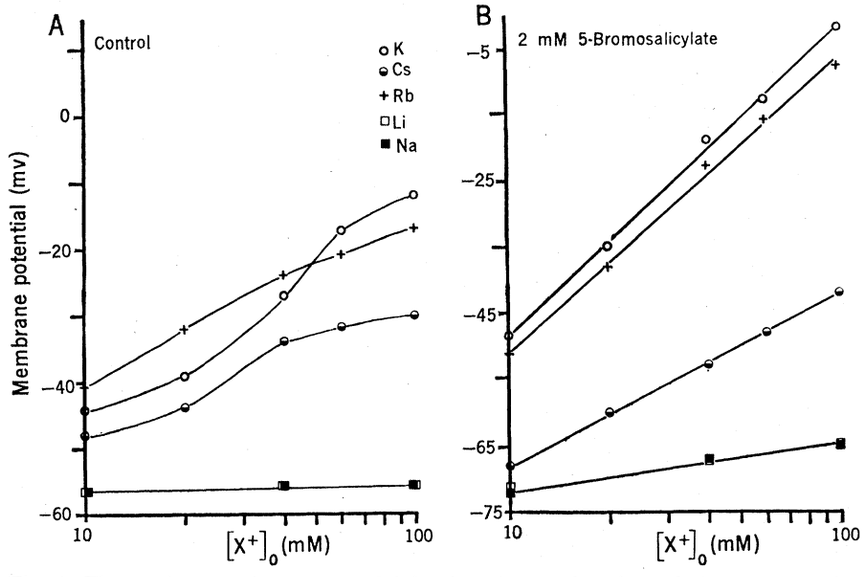

Fig. 1. The resting membrane potential is plotted as a function of the concentration and species of alkali cation present in the external medium under control conditions (A), and in the presence of 2 m*M* sodium 5-bromosalicylate (B). These examples from cell G-L are typical of the results obtained from all cells studied. Data points were generated by varying the cation species at each concentration. In drug-free salt solution (A), over a range of external cation concentration $[X^+]_0$ from 10 m*M* to 50 m*M*, a greater concentration of $[K^+]_0$ than $[Rb^+]_0$ is necessary to produce a given membrane potential. The $[Cs^+]_0$ required for a particular membrane potential is greater than both K^+ and Rb^+. The membrane potential is not dependent upon Na^+ or Li^+ over this concentration range. In the presence of 2 m*M* 5-bromosalicylate (B), more $[Rb^+]_0$ than $[K^+]_0$ was required to produce a given membrane potential, over the entire range of concentration studied. Furthermore, more $[Cs^+]_0$ than under control conditions was required to produce a given potential. These curves enable one to estimate the relative permeability of the membrane to the alkali cations; they demonstrate that salicylate has reduced Cs^+ and Rb^+ permeability relative to K^+.

anion permeability (primarily SO_4^{2-} in this case) caused by increased concentrations of external monovalent cations (*6, 11*). Calculations of relative cation permeability were made with reference to the membrane potential produced by $[K^+]_0$ in the range from 10 to 30 m*M* since these concentrations were close to the physiological K^+ concentration (10 m*M*), and cation effects on anion permeability were presumably minimal. For cation concentrations in this range, more external K^+ than Rb^+ was required to produce a given membrane potential under control conditions (Fig. 1A), an indication that P_{Rb} was greater than P_K in this concentration range. For example, to produce a membrane potential of −40 mv required a $[Rb^+]_0$ of 10 mmole/liter and a $[K^+]_0$ of 20 mmole/liter, so that P_{Rb}/P_K was 2.0. By analogy, the P_{Cs}/P_K ratio was calculated to be 0.67. Since the membrane was practically impermeable to Na^+ and Li^+ in this concentration range, values of P_{Na}/P_K were obtained by increasing the external concentration of these ions up to 500 mmole/liter (not illustrated). At this concentration the membrane potentials could be compared with those produced by K^+ at a concentration of 10 or 20 m*M* and the permeability ratios quantified. The relative permeability under control conditions was as follows: Rb^+ (1.38) > K^+ (1.0) > Cs^+ (0.71) > $Na^+ = Li^+$ (0.06) (these results are the average values for 23 cells at a $[K^+]_0$ of 20 mmole/liter).

The addition of a salicylate derivative to the bathing medium altered the relative cation permeability. In the presence of 5-bromosalicylate at a concentration of 2 mmole/liter (*12*) [this derivative has about 20 times the potency of salicylate itself (*3*)], the membrane potential varied linearly with the logarithm of the external cation concentration (Fig. 1B), and with greater slope, presumably because of reduced anion permeability (*2*). More Rb^+ than K^+ was required to produce a given membrane potential over the entire range of cation concentrations, an indication that the Rb^+ permeability was less than the K^+ permeability (Fig. 1B). In the case shown a membrane potential of −35 mv was produced by 20 mmole/liter of $[K^+]_0$, 24 mmole/liter of $[Rb^+]_0$, or 140 mmole/liter of $[Cs^+]_0$ (extrapolated), so that P_{Rb}/P_K was 0.75 and P_{Cs}/P_K was 0.15. Averaging the results of 12 cells at a $[K^+]_0$ of 20 mmole/liter, we found that the relative permeability in 2 to 3 m*M*/liter 5-bromosalicylate was as follows: K^+ (1.0) > Rb^+ (0.71) > Cs^+ (0.15) > $Na^+ = Li^+$ (<0.01).

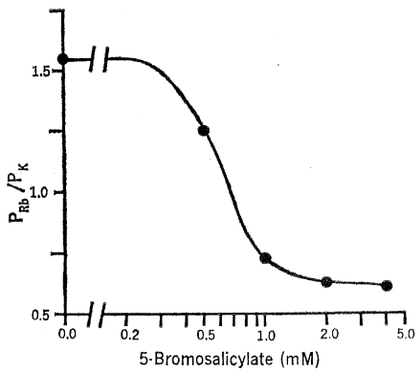

Fig. 2. Dose-response curve of the permeability of Rb^+ relative to K^+ as a function of the concentration of 5-bromosalicylate. Data points were obtained by determining the $[Rb^+]_0$ which would produce the same membrane potential as 20 m*M* $[K^+]_0$ in progressively increasing doses of salicylate.

The change in the order of selectivity caused by the addition of the salicylate represents a shift from Eisenman's transition sequence III to sequence IV, and implies an increase in the field strength of anionic sites in the membrane (*5, 6*). The effect of salicylate on cation selectivity was reversible, approaching control values 10 to 15 minutes after the drug had been washed out. For seven cells the average was as follows: K^+ (1.0) > Rb^+ (0.95) > Cs^+ (0.35) > $Na^+ = Li^+$ (0.01).

The change in the P_X/P_K ratio is a sigmoidal function of salicylate concentration, as the dose-response curve for P_{Rb}/P_K illustrates (Fig. 2). The concentration required to produce half the maximum change in the permeability ratio is approximately the same as that required to produce half the maximum change in membrane potential in more physiological salt solutions, so that the expression for the relative activity of salicylate derivatives as a function of their physiochemical parameters, developed previously with respect to the change in membrane potential (*3*), is also applicable for the change in P_X/P_K ratios.

In summary, the results indicate that salicylate derivatives reversibly alter the relative cation permeability of neuronal membranes. The direction of the change suggests that the underlying cause is an increase in the field strength of membrane anionic sites (*4, 5*), although other interpretations are possible (*13*). Since there is a high correlation between a molecule's ability to influence cation selectivity and its octanol-water partition coefficient, we propose that the effects of these anions on neuronal membranes can be accounted for simply in terms of their ability to adhere to these membranes and thereby change their anionic field strength.

Since the salicylate derivatives are only one example of drug congeners whose biological activity is highly correlated with their partition coefficients (*14*), it is possible that the basis for their action is the effect they exert on the permeability of biological membranes by influencing the field strength of charged membrane sites (*15*). We believe that this effect is the basis for the analgesic action of salicylate (*16*).

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7. Cells were identified according to the nomenclature of H. Levitan, L. Tauc, J. P. Segundo [*J. Gen. Physiol.* **55**, 484 (1970)]. Animals were obtained from Pacific Bio-Marine Supply Co., Venice, Calif.
8. We changed the external cation concentration by using the appropriate mixture of 620 m*M* tris sulfate and 100 m*M* $MgSO_4$ with 250 m*M* X_2SO_4, 100 m*M* $MgSO_4$, 130 m*M* mannitol (where X = Cs, K, Rb, Na, or Li). The *p*H of these solutions was 8.0, and the osmolarity was 860 milliosmoles.
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17. We thank Drs. H. Gainer, S. Rapoport, and P. Nelson for their comments, J. Lewis for photographic aid, and M. L. Adams for typing the manuscript.

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