OCCLUSION OF IONS BY THE Na-K-Cl COTRANSPORTER IN THE INTACT RECTAL GLAND OF THE SPINY DOGFISH, SQUALUS ACANTHIAS

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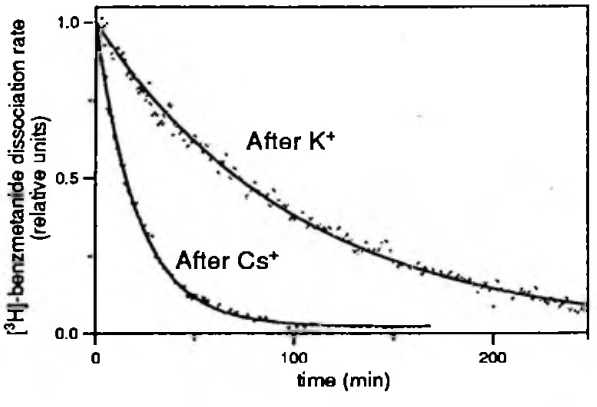
It is thought that in the process of moving ions across the membrane, a transport protein may go through conformational states in which the solute molecule is hidden within bilayer, inaccessable to solutions on both sides of the membrane. The existence of such "occluded" states has been clearly demonstrated for the Na pump (Glynn, I.M., and Richards, D.E. , J. Physiol. (Lond.), 330, 17-43, 1982; Forbush, B. III , J. Biol. Chem., 262, 11104-11115, 1987) and it has been proposed that occlusion may be a general feature of the operation of transporters (Forbush, B. III in The Na^+,K^+-pump, Skou, J.C., Norby, J.G., Maunsbach, A.B., and Esmann, M. eds., 229-248, Alan R. Liss, Inc., New York, 1988).

One of the hallmarks of solute occlusion is that the behavior of the protein may be affected by the presence of the occluded molecule, and it may thereby "remember" that a solute has been present long after that solute has been removed from solution. Using membranes prepared from shark rectal gland, we have previously reported that following inhibition of the Na-K-Cl cotransporter with [^{3}H]benzmetanide and removal of the inhibitor, the rate of dissociation of [^{3}H]benzmetanide depends on the ions that were present during binding, not on the composition of the medium during dissociation (Forbush, B. III, and Haas, M. , Biophys. J., 55, 422a, 1989); similar observations have recently been reported in membranes from rabbit parotid gland (Moore, M.L., George, J.N., and Turner, R.J. , Biochem. J., 309, 637-642, 1995).

In the present study we examined the rate of dissociation of [^{3}H]benzmetanide from Na-K-Cl cotransporters in the intact perfused gland (cf. Forbush, B. III, Haas, M., and Lytle, C. , Am. J. Physiol., 262, C1000-C1008, 1992). In each experiment we stimulated the rectal gland with VIP, and then perfused for a 3 min period with a solution containing Rb^+, NH_4^+, or Cs^+ in substitution for K^+; during this time we introduced [^{3}H]benzmetanide for a 1 min binding period. Excess radioactivity was then removed from the gland in a 10 minute perfusion period with shark Ringer's, following which we examined the amount of [^{3}H]benzmetanide which was released into the venous effluent at 1-5 min intervals for up to 5 hours in shark Ringer's. During the entire dissociation period, the perfusion solution contained 500 µM furosemide to prevent newly released [^{3}H]benzmetanide from rebinding to uninhibited transporters. VIP was also included to insure that transporters remained in an activated state, although similar dissociation rates were observed in the absence of VIP.



The result of a typical pair of experiments is illustrated in the figure. As shown here for Cs^+, there was a dramatic dependence of the [^{3}H]benzmetanide dissociation rate on the identity of the ion that had been present during association of [^{3}H]benzmetanide, even though the dissociation was monitored in the same solution in both experiments. Comparing K^+ substitutes (in three experiments each, except for NH_4^+), the influence on the subsequent dissociation rate of [^{3}H]benzmetanide was: $Cs^+ > NH_4^+ > Rb^+ > K^+$. The rate of dissociation was also 10-fold faster following binding in SCN compared to Cl. The absolute value of the rate constants (here 0.0084 min^{-1} following K^+, .042 min^{-1} following Cs^+) was about 2.5 fold lower than previously reported in isolated membranes, although the dependence upon ions was similar (Forbush, B. III, and Haas, M. , op cit). We presume this difference reflects the temperature difference between the two sets of experiments -- 15°C here compared to 20°C in the earlier experiments. Continuous perfusion proved to be a reproducible method for obtaining the dissociation rate: the SEM in triplicate determinations of dissociation rates was in each case less than ±4%.

These results show that the Na-K-Cl cotransporter can "remember" for several hours the ions which were present during a brief exposure to [^{3}H]benzmetanide. It seems most likely that this phenomenon occurs because the ions are trapped in the translocation pocket when the inhibitor is bound and that they can not escape until benzmetanide is released. A similar situation has been found with regard to ouabain binding by the Na pump (Forbush, B. III , Curr. Top. Membr. Trans., 19, 167-201, 1983). However unlike the Na pump, it has not yet been possible to obtain evidence that the uninhibited Na-K-Cl cotransporter occludes ions.

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