THE BINDING SITES INVOLVED IN p-CHLOROMERCURIBENZENE SULFONATE (PCMBS) INDUCED SWELLING OF DOGFISH (SQUALUS ACANTHIAS) RECTAL GLAND CELLS

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Slices of dogfish rectal gland lose K^+, gain Na^+ and Cl^-, and swell when exposed to PCMBS. Previously, we showed that PCMBS increases membrane permeability to cations, and inhibits the Na-K-ATPase (Kleinzeller et al., MDIBL Bull. 26:163, 1986). We report here studies aimed at defining the location and nature of the chemical groups with which PCMBS reacts. Experimental methods are described elsewhere (Kleinzeller et al., loc. cit.; Ziyadeh et al., this Bull.).

1 mM PCMBS produces swelling after a lag of 30 to 60 min. The membrane might act as a barrier to diffusion of the PCMBS anion. A comparison of swelling seen with PCMBS to that produced by 1 mM mersalyl or the more lipid soluble mercurials, p-hydroxymercuribenzoate (PHMB) and phenylmercuric acetate (PMA), indicates that the explanation is more complex. At 2 h, the swelling effect was: PCMBS = mersalyl > PMA > PHMB. As with PCMBS, swelling with PHMB occurs after a delay. The rates of swelling with PCMBS and PHMB during the 2nd h are comparable; with PMA, it is 60% less. The rate with mersalyl is similar to that with PCMBS; however, the pattern of cation changes with mersalyl differs from that with PCMBS in that the uptake of Na^+ is more and the loss of K^+ is less, and occur only in the 1st h. Thus, the two mercurials may have different target sites. In addition, mersalyl and PMA do not reduce PCMBS uptake, as expected if they competed for binding sites (see below).

Uptake of [203Hg]-PCMBS was followed in slices rinsed 2 min in saline with no PCMBS to eliminate a contribution of the extracellular space. With 0.01 mM, uptake is rapid the first 15 min; there is some further uptake, saturating by 30 min (35 ± 8 nmol/g tissue dry wt.) [mean ± SE, n = 4]. Uptake of either 0.1 or 1 mM PCMBS shows no saturation over 2 h, with an accumulation of the mercurial against an apparent gradient (2.5 at 0.1 mM PCMBS, 1.2 at 1 mM). The pattern of uptake with 0.1 and 1 mM PCMBS may represent rapid interaction with superficial sites, followed by permeation through the membrane and binding to deeper sites, as found in RBC (Knauf and Rothstein, J. Gen. Physiol. 58: 211, 1971). No evidence was found for a carrier mediated uptake. 0.1 mM bumetanide, 0.5 mM ouabain and 0.1 mM DIDS, which reduces PCMBS uptake by RBC, have no effect on 1 mM PCMBS uptake at 30 min. The latter two do reduce uptake at 120 min by 12 and 26%, respectively; this reduced uptake is associated with reduced swelling. Manipulations which cause additional swelling, i.e., replacement of Na^+ with Li^+ or K^+, or the addition of another mercurial or N-eithylmaleimide (NEM), are associated with additional uptake. Thus, the pattern of 1 mM PCMBS uptake over 2 h may reflect induced exposure of additional binding sites. Such cooperativity between binding and uptake would also help to explain the lag seen in PCMBS-induced swelling.

The presence of two binding sites in series is supported by analysis of PCMBS efflux from slices loaded 1 h in 1 mM PCMBS. Efflux can be resolved into two cellular components: a fast (time constant of 0.153 min^{-1}), and

slow (0.0067 min^{-1}), their relative pools being 46% and 30%. Binding is not tight; only some 30% of PCMBS remains in the tissue after 1 h. The effect of membrane permeant dithiothreitol (DTT) indicates that PCMBS is indeed bound. With 1 mM DTT, only some 9% of PCMBS remains at 1 h. DTT increases the time constant of the slow component (to 0.023 min^{-1}), with no effect on the fast component. The impermeant metallothionein (10 ug/ml) has no effect on efflux. Thus, the fast cellular component likely represents superficial PCMBS, and the slow component deeply bound PCMBS (accessible to DTT, but not metallothionein).

At low concentrations, PCMBS reacts exclusively with sulfhydryls; at concentrations exceeding the number of titratable sulfhydryls, PCMBS may react with other groups (Rothstein, Curr. Top. Membr. Transp. 1:135, 1970). The membrane permeant, sulfhydryl-specific reagent, sodium tetrathionate (TET) was used to assess this possibility. TET by itself has no effect on tissue water or cations. Pretreatment with 20 mM TET does not affect the amount of swelling with PCMBS at 2 h, whereas cotreatment reduces it by 50% (Fig. 1). Cotreatment has only a slight effect on K^+ loss, reduced swelling being due to a marked reduction in Na^+ uptake. Thus, part of the mechanism underlying PCMBS-induced K^+ loss may not involve SH groups. This would account in part for why NEM, a membrane permeant, sulfhydryl alkylating agent, does not block the action of PCMBS. Rather, pre- or cotreatment with 1 mM NEM enhances PCMBS swelling (Fig. 1). This may be because NEM inhibits the Na-K-ATPase. At 2 h, NEM decreases K^+ 34% and increases Na^+ 18%, with no swelling. Ouabain for the same length of time has a similar effect. Also, NEM does not affect 86-Rb efflux, further evidence that it causes K^+ loss by inhibiting the pump, and that sulfhydryls are not involved in determining K^+ permeability. Results with NEM, however, must be interpreted with caution, since not all sulfhydryls may be susceptible to NEM (Toon and Solomon, Biochim. Biophys. Acta 860:361, 1986). Several Bis-imidate esters, crosslinking agents that react with amino groups, had no effect on PCMBS swelling or uptake. However, H_2DIDS, which reacts with lysine groups, reduces PCMBS uptake and swelling 13%.

PCMBS acts on the uptake and efflux of taurine, a major intracellular osmolyte (50 mM) in the shark rectal gland. 0.1 and 1 mM PCMBS inhibit 0.2 mM [^{14}C]-taurine uptake by 33 and 50%, respectively. One likely mechanism is the dissipation of transcellular Na-gradient necessary for Na-dependent taurine uptake. Pretreatment of tissue with 0.1 mM PCMBS does not affect the subsequent uptake of taurine in control media, reflecting the loss of mercurial from the tissue. The efflux of taurine from labeled tissue increases 7 fold in 1 mM PCMBS compared with the control. This effect is not due to cell swelling in PCMBS; increasing cell volume in propionate medium (Feldman et al., this Bull.) is without effect on taurine efflux.

These results indicate that there are several types of binding sites for PCMBS in the shark rectal gland. The Na-K-ATPase appears to be one target. Mercurials also affect the cell permeability for cations and cell structure (Kleinzeller et al., MDIBL Bull. 26:163, '86) and cellular levels of osmolytes, taurine and myo-inositol (McGregor and Kleinzeller, MDIBL Bull., 26:168, 1986). The role of membrane permeability in mediating the observed effects of various mercurials, and their action on tissue -SH groups will have to be investigated quantitatively.

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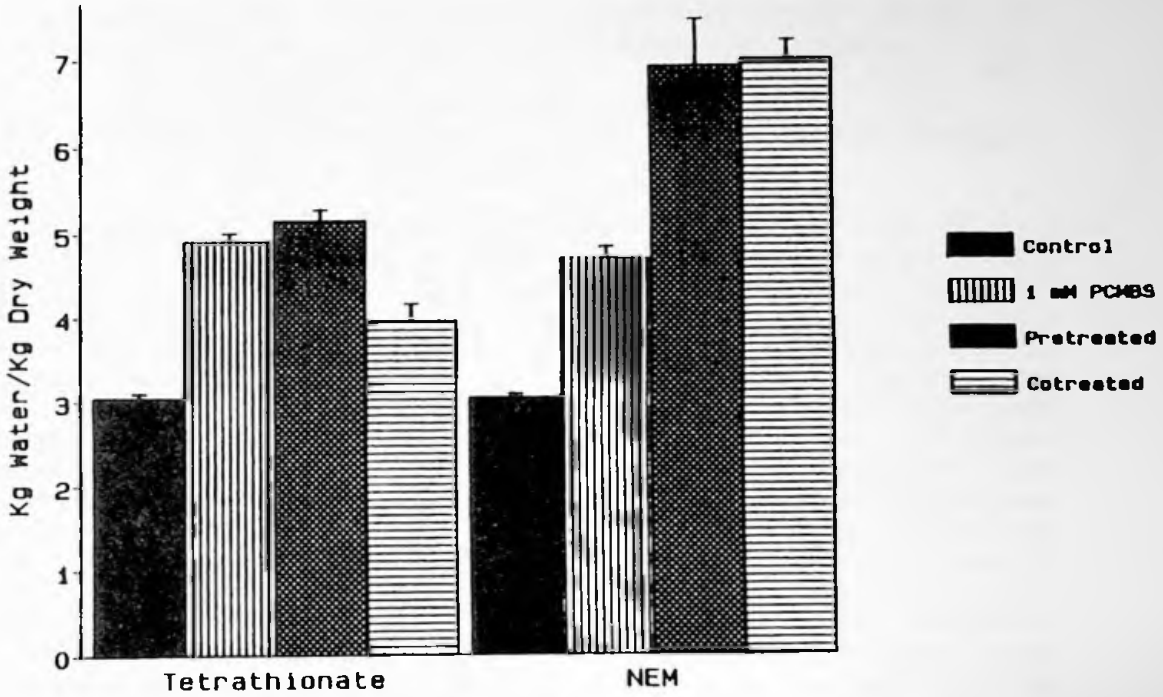

Fig. 1. Swelling effects of PCMBS, TET and NEM on rectal gland slices. Tissue was either pretreated with TET or NEM, and subsequently the effect of PCMBS was studied in the absence of the two agents; alternatively, tissue was first treated with the thiol reagents, and then PCMBS was added (cotreatment).