COMPARISON OF LEAD AND BARIUM EFFECTS ON ELECTROPHYSIOLOGIC PROPERTIES OF THE IN VITRO PERFUSED DILUTING SEGMENT FROM THE PERITUBULAR SHEATH OF SQUALUS ACANTHIAS KIDNEY

Steven C. Hebert and *Peter A. Friedman. Departments of Medicine and Physiology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA; *Department of Pharmacology and Toxicology, Dartmouth Medical School, Hanover, NH.

We have recently identified a diluting segment in the dogfish kidney which is localized to the peritubular sheath region of the nephron (Friedman & Hebert, Bull. MDIBL 25:24-26,1985;Hebert & Friedman, 25:128-131, 1985 and 26: ??-??, 1986). This segment of the dogfish nephron has many of the characteristics of diluting segments from both amphibia and mammals (Hebert and Andreoli, Am.J.Physiol. 246:F745-F756, 1984). Specifically, NaCl absorption by these diluting segments is mediated by a secondary active transport mechanism dependent on basolateral membrane Na-K-ATPase and involves a furosemide-sensitive, apical membrane $Na^{+}:K^{+}:2Cl^{-}$ cotransporter. In addition, we and others (see Hebert & Andreoli, Am.J.Physiol. 246:F745, 1984 for a review; Hebert & Friedman, 26:??-??, 1986) have shown that the equivalent short circuit current [Isc ($\mu A/cm^2$) = transepithelial voltage (Ve, mV) times transepithelial conductance (Ge, mS/cm^2)] in these diluting segments is a good approximation of the net rate of NaCl absorption. For a 1:1:2 stoichiometry of the apical membrane cotransporter, 50% of Na^+ absorbed traverses the transcellular route and the other half of Na^+ absorption is driven through the Na^+-permselective paracellular pathway by the lumen-positive Ve.

Diluting segments are also characterized by apical membranes which are virtually exclusively conductive to K^+. This pathway (i) provides a route for the transcellular conductance which contributes 20-60% of the total transepithelial conductance; (ii) allows for recycling back across this membrane the K^+ that entered the cell via the cotransporter; (iii) permits net current flow across the cell which under open circuit conditions returns via the Na^+-permselective paracellular route; and (iv) is crucial to the generation of the lumen-positive transepithelial voltage. This K^+ conductance in diluting segments from mammals and amphibia can be blocked by millimolar concentrations of Ba^{++}. Consequently, Ba^{++} addition to luminal (apical) solutions reduces or abolishes Ve, Ge and Isc.

Lead is one of the major environmental toxins known to contaminate water supplies in the U.S.A. The adverse effects of lead on red blood cells and neural tissue have been well described; however, little is known about how this metal effects (if at all) epithelial function. The purpose of the present study was to investigate the effects of lead on the electrophysiologic properties of the diluting segment from the peritubular sheath of the dogfish, *Squalus acanthias*, kidney and to compare these lead effects with those of another agent, barium, which is known to alter the electrophysiologic parameters related to sa transport in diluting segments (Hebert & Friedman, Bull. MDIBL 25:128-131,1985; Hebert & Andreoli, Am.J.Physiol. 246:F745-F756, 1984).

MATERIALS AND METHODS: Diluting segments from male 2-4 kg dogfish, *Squalus acanthias*, were perfused *in vitro* using methods similar to those described by us for the mammalian thick ascending limb (Hebert et al., J.Membr.Biol. 80:201-219, 1984) and previously for the dogfish (Hebert & Friedman, Bull.MDIBL 25:128-131, 1985). Tubule segments, 0.3-0.4 mm in length, were dissected free-hand from peritubular sacs located in the bundle zone and identified by the characteristic large outer diameter and "cobblestone" appearance of the cells (Hebert & Friedman Bull.MDIBL 26:??-??, 1986). All tubules were perfused at 16-18°C using symmetrical shark Ringers. Ve was measured lumen with respect to bath. Command current pulses, 600 nA in magnitude and 400 ms in duration, were delivered from an IBM computer through a D/A converter to a current clamp amplifier. Voltage deflections at the perfusion and collecting ends of the tubule were digitized and stored on floppy disks. Ge was calculated from terminated cable equations (Hebert et al. J.Membr.Biol. 80:201-219, 1984) and Isc=Ge•Ve. Results are presented as mean values ± SEM.

RESULTS AND DISCUSSION: The effects of luminal one millimolar Pb^{++} or Ba^{++} on the electrical properties of the dogfish diluting segment are shown in Table 1. While both cations reduced the transepithelial conductance by a similar fraction (30% for Ba^{++} and 38% for Pb^{++}), their effects on Ve and Isc were quite different. Ba^{++} reduced both Ve and Isc, typical of blockade of the apical cell membrane K^{+} conductance in diluting segments (Hebert & Andreoli, Am.J.Physiol. 246:F745, 1984); however, a similar concentration of Pb^{++} resulted in a significant rise in Ve such that the Isc (i.e., Ve•Ge) was unchanged. In other words, the rise in Ve associated with luminal Pb^{++} addition maintained Isc at the control value despite the fall in Ge.

The effects of Pb^{++} on Ge and Isc for the concentration range 1-1000 µM are shown in Figure 1. Control values for Ge and Isc in these four diluting segments were 103.2 ± 15.4 mS/cm² and 1092 ± 530 µA/cm², respectively. It is clear that luminal Pb^{++} produced a concentration-dependent reduction in Ge, but had no significant effect on Isc (although Isc tended to fall slightly at 1000 µM). In addition, there is a linear relationship between the log Pb^{++} concentration and the transepithelial conductance (least squares fit. $Ge = -11 \bullet Log[Pb^{++}] + 31.8$, r=0.99).

The effects of Ba^{++} or Pb^{++} on the transepithelial voltage responses at the perfusion end of the tubule to square-wave current pulses are shown in Figure 2a-d. The control responses are shown in 2a and 2c for Ba^{++} and Pb^{++}, respectively. Note that there is a small creep (polarization effect) in the voltage over time after an initial large voltage defection. This voltage "creep" is typical of epithelia with large apical K^{+} conductances and is abolished by Ba^{++}-mediated blockade of the K^{+} conductance (Hebert et al., J.Membr.Biol. 80:201-219, 1984). Thus the "squaring off" of the voltage deflection shown in Figure 2b is consistent with a Ba^{++}-mediated blockade of an apical cell membrane K^{+} conductive pathway in the dogfish diluting segment. On the other hand, luminal Pb^{++} had no discernable effect on this "creep" voltage (Fig. 2d).

In summary, the electrical effects of luminal Pb^{++} over the range 1-1000 µM are characterized by: (i) a concentration-dependent reduction in Ge (Fig. 1) which is similar in magnitude (at one mM) to that produced by the K^{+}-channel blocker, Ba^{++} (Table 1); (ii) maintenance of Isc due to a concentration-dependent rise in Ve (Table 1; Figs. 1-2); and (iii) a transepithelial voltage response to current injection which is not consistent with an effect of Pb^{++} on an apical K^{+} conductance pathway. These observations suggest that Pb^{++} may be selectively reducing the cation-selective paracellular (shunt) pathway. Further experiments will be required to verify this possibility.

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TABLE 1. COMPARISON OF 1 mM LEAD AND BARIUM ON THE ELECTROPHYSIOLOGIC PROPERTIES OF THE DILUTING SEGMENT FROM THE PERITUBULAR SHEATH

Agent	Ve (mV)	Ge (mS/cm^2)	Isc ($\mu A/cm^2$)	n
Control	10.6 ± 2.2	103.2 ± 15.4	1080 ± 270	8
Pb^{++}	*14.4 ± 6.6	*64.0 ± 6.3	875 ± 320	4
Control	5.2 ± .08	81.0 ± 6.7	540 ± 110	4
Ba^{++}	*2.3 ± .05	*57.1 ± 7.1	*130 ± 40	4

Tubules were perfused *in vitro* at 16-18°C using symmetrical shark ringers. Data are expressed as mean ± SEM. * indicates $p < .05$ compared to the control value.

Figure 1. Concentration dependent effects of luminal lead on the transepithelial conductance (Ge; see 1A) and the short circuit current (Isc; see 1B) in the dogfish diluting segment from the peritubular sheath. Tubules were perfused and bathed at 16-18°C using symmetrical shark ringers. Ve was measured lumen with respect to bath. Isc = Ve•Ge, where Ge was calculated from terminated cable equations using hyperpolarizing current pulses.

FIG. 1A.

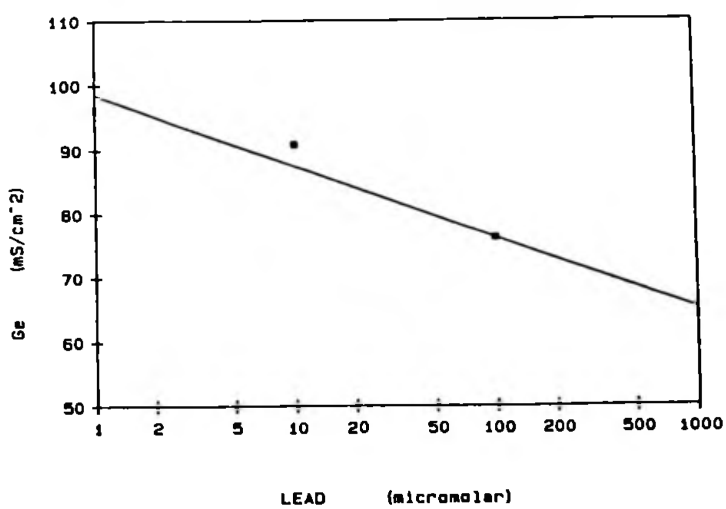

FIG. 1B.

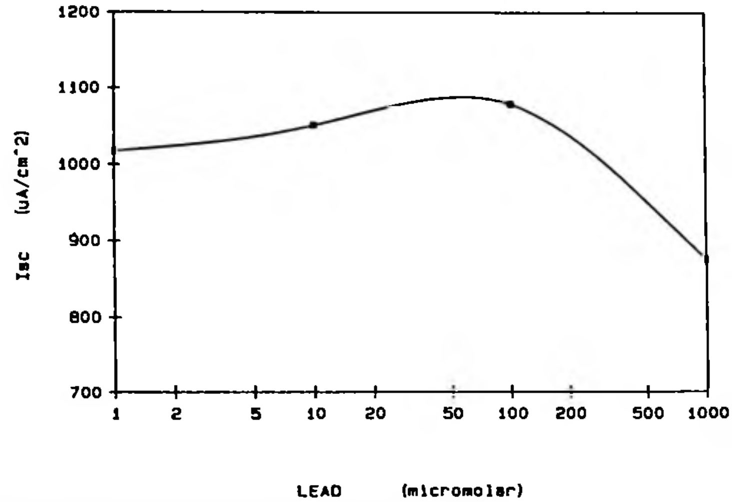

FIGURE 2. Representative voltage deflections at the perfusing pipet for square-wave current pulses in the presence and absence of either 1 mM barium or 1 mM lead. Shark diluting segments from the peritubular sheath were perfused and bathed *in vitro* at 16-18°C using symmetrical shark ringers. 2A, Control before Ba^{++}; 2B, one minute after adding 1 mM Ba^{++} to the perfusate; 2C, control before lead; 2D, 5 minutes after adding 1 mM lead to the perfusate. Upward voltage deflections are positive for hyperpolarizing current pulses.