Effect of Bromotetramisole on Renal Phosphate Excretion (44050)

MARCINE ONSGARD-MEYER, ANGELA L. MCCOY, AND FRANKLYN G. KNOX[1]
Departments of Medicine and Physiology & Biophysics, Mayo Clinic and Foundation, Rochester, Minnesota 55905

Abstract. **Levamisole inhibits alkaline phosphatase (ALP) activity in kidney brush border membranes and increases phosphate excretion *in vivo* in dogs and rats. *l*-p-Bromotetramisole (*l*-BR) is a more potent analog of levamisole in regard to inhibition of ALP activity *in vitro*, but had no effect on phosphate transport by *in vitro* proximal tubules of the rabbit. Since its effect on phosphate excretion *in vivo* has not been studied, the present study tested the effects of infusion of *l*-BR on phosphate excretion in Sprague-Dawley rats. Fractional excretion of phosphate (FE_{PI}) was measured in thyroparathyroidectomized Sprague-Dawley rats before and during a systemic infusion at 0.8 ml/min of 10 m*M* *l*-p-Bromotetramisole oxalate (*l*-BR, $n = 6$), or the inactive isomer *d*-p-Bromotetramisole oxalate (*d*-BR, $n = 5$). The FE_{PI} increased significantly from 4.7% ± 0.9% to 13.4% ± 3.1% in response to *l*-BR whereas there were no changes in FE_{PI} with inactive *d*-BR. In conclusion, systemic infusion of *l*-p-Bromotetramisole increases FE_{PI} in Sprague-Dawley rats.** [P.S.E.B.M. 1996, Vol 213]

Increased alkaline phosphatase (ALP) activity in the brush border membranes of proximal tubules (BBMV) has been correlated with increased phosphate uptake (1). Inhibition of ALP with levamisole has been shown to decrease phosphate uptake in brush border membrane vesicles (BBMV) of the rat and dog, and infusion of levamisole increased phosphate excretion *in vivo* (2). *l*-p-Bromotetramisole (*l*-BR) is an analog of levamisole which is reported to be more potent than levamisole (3) and has been shown to inhibit ALP activity in many tissues including the brush border membranes of the rat kidney (4, 5). However, surprisingly, *l*-BR did not change phosphate transport in isolated perfused proximal convoluted tubules of the rabbit (6). The *in vivo* effect of *l*-BR on phosphate excretion has not been studied. Therefore, the objective of the present study was to determine whether a systemic infusion of *l*-BR would increase the urinary excretion of phosphate in Sprague-Dawley rats. The inactive isomer of *l*-BR, *d*-p-Bromotetramisole (*d*-BR), was used as a control.

Materials and Methods

Experiments were performed on adult male Sprague-Dawley rats weighing 275–350 g (Harlan Sprague-Dawley, Indianapolis, IN). Rats were maintained on a normal phosphate diet (ICN low-phosphate diet supplemented with mono and dibasic sodium and potassium phosphate, NaCl and K_2CO_3; 0.7% phosphorus, 1.0% calcium) and water *ad libitum* for 2 days prior to the experiment, and were monitored to insure ingestion of food daily. Rats were anesthetized with an intraperitoneal injection of 100 mg/kg body wt Inactin (Byk-Gulden, Konstanz, West Germany) and placed on a heated table to maintain body temperature between 36° and 38°C. Rats were acutely thyroparathyroidectomized (TPTX) by heat cautery, and a tracheostomy was performed. A PE 240 catheter was placed in the trachea to facilitate spontaneous breathing. Catheters (PE 50) were placed in the left carotid artery for mean arterial pressure measurement (MAP) and blood sampling, into both jugular veins for infusions, and into the bladder (PE 90) for urine collection. An infusion of 3% inulin dissolved in a 1% albumin-saline solution was initiated at a rate of 3.3 ml/hr and continued throughout the experiment. In addition, 0.9% sa-

[1] To whom requests for reprints should be addressed at Departments of Medicine and Physiology & Biophysics, Mayo Clinic and Foundation, 200 First Street SW, Rochester, MN 55905.

Received February 12, 1996. [P.S.E.B.M. 1996, Vol 213]
Accepted May 20, 1996.

This work was supported by National Institutes of Health Grant DK 19715.

0037-9727/96/2132-0193$10.50/0

line was infused at 2.5 ml/hr for the first hour, then decreased to 0.8 ml/hr for the remainder of the experiment. In each rat, a 30-min control clearance was collected at least 2 h after TPTX. After the control clearance, 10 m*M* *l*-Bromotetramisole oxalate (*l*-BR) (n = 6) or inactive *d*-Bromotetramisole oxalate (*d*-BR) n = 5) (Aldrich Chemical Co., Milwaukee, WI) replaced the saline infusion and was infused at 0.8 ml/hr. The Bromotetramisole was dissolved in normal saline and was adjusted to a pH of 5.0–6.5 with NaOH to prevent precipitation. A 10-min equilibration period was followed by a 45-min clearance period. A blood sample of approximately 0.5 ml was collected at midpoint of each clearance.

Glomerular filtration rate (GFR) was determined by measurement of the renal clearance of inulin, measured by the anthrone method (7). Concentrations of sodium and potassium in urine and plasma were measured by flame photometry (Instrumentation Laboratories, Lexington, MA), phosphate by the method of Chen (8), and urinary 3′5′ cyclic adenosine-monophosphate (cAMP) by radioimmunoassay (Biomedical Technologies, Stoughton, MA). Statistical comparisons were made using a Student's t test, with a p value designated as less than 0.05.

Results

That data are summarized in Table I. Infusion of *l*-BR significantly increased fractional excretion of phosphate (FE_{Pi}) from 4.7% ± 0.9% to 13.4% ± 3.1% ($P < 0.05$). Plasma phosphate (P_{Pi}) increased significantly with *l*-BR. Fractional excretion of sodium (FE_{Na}) tended to increase with *l*-BR infusion, (1.3% ± 0.3% to 2.1% ± 0.4%); however, this did not reach statistical significance. MAP and GFR did not change significantly throughout the experiment. Urinary cAMP was also unchanged with infusion of *l*-BR. Infusion of the inactive isomer, *d*-BR, did not change FE_{Pi} significantly (5.2% ± 2.2% to 7.7% ± 2.8%), but FE_{Na} increased significantly with infusion of *d*-BR (1.0% ± 0.5% to 3.7% ± 0.2%, $P < 0.05$). MAP and GFR remained stable with *d*-BR, and likewise P_{Pi} and cAMP did not change significantly.

Table I. Effect of the Systemic Infusion of l-BR (n = 6) or *d*-BR (n = 5)

	Control	BR
MAP (mm Hg)		
l-BR	113 ± 6	113 ± 6
d-BR	119 ± 7	112 ± 5
GFR (ml/min)		
l-BR	2.4 ± 0.2	1.8 ± 0.2
d-BR	2.4 ± 0.3	2.2 ± 0.1
FE_{Pi} (%)		
l-BR	4.7 ± 0.9	13.4 ± 3.1[a]
d-BR	5.2 ± 2.2	7.7 ± 2.8
FE_{Na} (%)		
l-BR	1.3 ± 0.3	2.1 ± 0.4
d-BR	1.0 ± 0.5	3.7 ± 0.2[a]
P_{Pi} (m*M*)		
l-BR	3.4 ± 0.1	3.7 ± 0.2[a]
d-BR	3.4 ± 0.2	3.0 ± 0.4
cAMP (pg/ml/min)		
l-BR	45.3 ± 7.2	45.0 ± 7.1
d-BR	43.2 ± 3.4	43.0 ± 5.9

Note. Values are expressed as mean ± SEM. n, number of animals; GFR, glomerular filtration rate; MAP, mean arterial pressure; FE_{Pi}, fractional excretion of phosphate; P_{pi}, plasma phosphate concentration; cAMP, cyclic 3′5′ adenosine-monophosphate.

[a] Significant difference from control, $P < 0.05$, Student's t test.

Discussion

In the present study, systemic infusion of *l*-BR resulted in a significant increase in the FE_{Pi} in Sprague-Dawley rats. Since there were no increases in filtered load, these results suggest that *l*-BR decreases phosphate reabsorption. These findings are consistent with a previous study, by PetitClerc *et al.*, who demonstrated that *in vivo* inhibition of ALP with infusion of levamisole decreased the fractional reabsorption of phosphate in rats and dogs (2).

Alkaline phosphatase is concentrated in the brush border membrane of the proximal tubule (4). Kempson *et al.* demonstrated increased ALP activity in the brush border membranes of rats adapted to low-phosphate diet, a condition where phosphate uptake is increased (1). However, Yusufi *et al.*, from the same laboratory, reported that the presence of ALP in BBM is not required for sodium gradient dependent transport of phosphate. They concluded that their findings argue against a direct involvement of ALP in sodium-dependent phosphate transport across the renal brush border membranes, but did not exclude the ALP might play a role in the modulation of phosphate transport (9).

Several *in vitro* studies performed in proximal convoluted tubules and in brush border membrane vesicles from proximal tubules (BBMV) which used various inhibitors of ALP, including *l*-p-Bromotetramisole, have not demonstrated a direct correlation between ALP activity and phosphate transport (6, 9–12). Brunette *et al.* inhibited ALP activity in rat BBMV with *l*-BR, but failed to demonstrate a decrease in phosphate uptake (6).

The discrepancy between the effects of inhibition of ALP *in vitro* and *in vivo* may be attributable to metabolic changes that may secondarily alter phosphate reabsorption. PetitClerc and Plante concluded that the phosphaturia induced by levamisole does not appear to be mediated by changes in parathyroid hormone secretion or other extrarenal hormonal substance, since the phosphaturia was seen when the drug was administered in the renal artery of the dog (2).

Similarly, the present experiments were performed in TPTX rats, again demonstrating that the phosphaturia produced during infusion of *l*-BR is independent of the effects of PTH. Alternatively, *l*-BR may cause nonspecific changes in phosphate transport independent of its effects as an inhibitor of alkaline phosphatase.

Considering the present results in the context of the literature, it is highly unlikely that ALP is a component or directly linked to sodium dependent phosphate transport across the brush border membrane of proximal tubules. Since ALP inhibitors are phosphaturic when directly administered to the intact kidney and the effect can be demonstrated in the absence of parathyroid hormone, we postulate that ALP plays a role in the modulation of the autocrine/paracrine regulation of phosphate transport by renal proximal tubule cells (13).

In conclusion, systemic infusion of *l*-Bromotetramisole increased the fractional excretion of phosphate in TPTX Sprague-Dawley rats.

1. Kempson SA, Kim JK, Northrup TE, Knox FG, Dousa TP. Alkaline phosphatase in adaptation to low dietary phosphate intake. Am J Physiol **237**:E465–E473, 1979.
2. PetitClerc C, Plante GE. Renal transport of phosphate: role of alkaline phosphatase. Can J Physiol Pharmacol **59**:311–323, 1989.
3. Borgers, M. The cytochemical application of new potent inhibitors of alkaline phosphatases. J Histochem Cytochem **21**:812–824, 1973.
4. Borgers M, Thoné F. The inhibition of alkaline phosphatase by *l*-p-Bromotetramisole. Histochemistry **44**:277–280, 1975.
5. Van Belle H, De Broe ME, Wieme RJ. *l*-p-Bromotetramisole, a new reagent for use in measuring placental or intestinal isoenzymes of alkaline phosphatase in human serum. Clin Chem **23**:454–459, 1977.
6. Brunette MG, Dennis VW. Effects of *l*-Bromotetramisole on phosphate transport by the proximal renal tubule: Failure to demonstrate a direct involvement of alkaline phosphatase. Can J Physiol Pharmacol **60**:276–281, 1982.
7. Führ J, Kaczmarczyk J, Krüttgen CD. Ein einfache colorimetrische Methode zur inulinbestimmung für Nierenclearanceuntersuchungen bei Stoffwechselgesunden und Diabetikern. Klin Wochenschr **33**:729–730, 1955.
8. Chen P, Toribara T, Warner H. Microdetermination of phosphorus. Anal Chem **28**:1756–1758, 1956.
9. Yusufi ANK, Low MG, Turner ST, Dousa TP. Selective removal of alkaline phosphatase from renal brush-border membrane and sodium-dependent brush-border membrane transport. J Biol Chem **258**:5696–5701, 1983.
10. Tenenhouse HS, Scriver CR, Vizel EJ. Alkaline phosphatase activity does not mediate phosphate transport in the renal cortical brush-border membrane. Biochem J **190**:473–476, 1980.
11. Letellier M, Plante GE, Brieére N, PetitClerc C. Participation of alkaline phosphatase in the active transport of phosphates in brush border membrane vesicles. Biochem Biophys Res Commun **108**:1394–1400, 1982.
12. Storelli C, Murer H. On the correlation between alkaline phosphatase and phosphate transport in rat renal brush border membrane vesicles. Pflugers Arch **384**:149–153, 1980.
13. Beers KW, Thompson MA, Berndt TJ, Onsgard-Meyer MJ, Knox FG, Dousa TP. Serotonin (5-HT) counter-regulates the inhibitory effect of dopamine on Na^+-phosphate cotransport in OK cells. J Am Soc Nephrol **6**:944, 1995.