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Effect of divalent cation ionophore (A 23187) on renal handling of phosphorus

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Effect of divalent cation ionophore (A 23187) on renal handling of phosphorus. To evaluate the effect of an increase in intracellular calcium on renal handling of phosphorus, calcium ionophore, which facilitates passive entry of calcium into cytosol, was given i.v. to four groups of rats: group 1, animals with intact parathyroid glands; group 2, parathyroidectomized (PTX) rats; group 3, PTX animals receiving i.v. parathyroid hormone (PTH); and group 4, PTX animals pretreated with i.v. ionophore, then given i.v. PTH. During the administration of ionophore in group 1, serum calcium (S_{Ca}) decreased from 8.7 ± 0.2 (mean ± SEM) to 7.5 ± 0.2 mg/100 ml ($P < 0.001$), fractional excretion of phosphorus (C_P/C_{In}) decreased from 0.110 ± 0.020 to 0.019 ± 0.006 ($P < 0.001$), and urinary cyclic 3′, 5′-adenosine monophosphate (U_{cAMP}) decreased from 131 ± 23 to 46 ± 16 pmoles/min ($P < 0.0125$). In group 2, during the administration of ionophore, S_{Ca} decreased from 6.5 ± 0.2 to 5.7 ± 0.2 mg/100 ml ($P < 0.001$), but neither C_P/C_{In} nor U_{cAMP} were altered. In group 2, during the administration of ionophore, C_P/C_{In} decreased from 0.43 ± 0.05 to 0.19 ± 0.04 ($P < 0.005$), U_{cAMP} decreased from 254 ± 20 to 159 ± 11 ($P < 0.001$). In group 4, during combined i.v. administration of ionophore and PTH, C_P/C_{In} was reduced from 0.19 ± 0.009 to 0.044 ± 0.012 ($P < 0.005$), and serum calcium was reduced from 6.5 ± 0.3 to 5.1 ± 0.3 mg/100 ml ($P < 0.01$). These findings indicate that i.v. ionophore suppresses urinary excretion of phosphorus, only in the presence of either endogenous or exogenous PTH. The associated decrease in U_{cAMP} suggests that this effect could be mediated through inhibition of PTH-dependent formation of cAMP, possibly resulting from the ionophore-induced increase in intracellular calcium in renal tubular cells.

Effet d'un ionophore des cations divalents (A 23187) sur le comportement renal vis a vis du phosphate. Afin d'évaluer l'effet de l'augmentation du calcium intracellulaire sur le comportement rénal vis à vis du phosphore, on a donné à quatre groupes de rats un ionophore de calcium qui facilite l'entrée passive de ce cation dans le cytosol. Le groupe 1 est composé d'animaux dont les parathyroïdes sont intactes, le groupe 2 d'animaux parathyroïdectomisés (PTX), le groupe 3 d'animaux PTX recevant de l'hormone parathyroïdienne (PTH) par voie intra-veineuse et le groupe 4 d'animaux pré-traités par l'ionophore qui reçoivent de la PTH. Au cours de l'administration de l'ionophore au groupe 1, le calcium séreique (S_{Ca}) diminue de 8,7 ± 0,2 (m̄ ± SEM) à 7,5 ± 0,2 mg/100 ml ($P < 0,001$), l'excrétion fractionnelle du phosphore (C_P/C_{In}) diminue de 0,110 ± 0,020 à 0,019 ± 0,006 ($P < 0,001$) et l'AMP cyclique urinaire (U_{cAMP}) diminue de 131 ± 23 à 46 ± 16 pmoles/min ($P < 0,0125$). Dans le groupe 2, au cours de l'administration de l'ionophore, S_{Ca} diminue de 6,5 ± 0,2 à 5,7 ± 0,2 mg/100 ml ($P < 0,001$), mais ni C_P/C_{In} ni U_{cAMP} ne sont modifiés. Dans le groupe 3, au cours de l'administration de l'ionophore, C_P/C_{In} diminue de 254 ± 20 à 159 ± 11 ($P < 0,001$). Dans le groupe 4, au cours de l'administration combinée d'ionophore et de PTH, C_P/C_{In} diminue de 0,19 ± 0,009 à 0,004 ± 0,012 ($P < 0,005$) et S_{Ca} de 6,5 ± 0,3 à 5,1 ± 0,3 mg/100 ml ($P < 0,01$). Ces constatations indiquent que l'ionophore ne diminue l'excrétion urinaire de phosphate qu'en présence de PTH endogène ou exogène. La baisse associée de U_{cAMP} suggère que cet effet peut avoir pour médiateur l'inhibition de la formation d'AMP cyclique dépendante de la PTH, conséquence de l'augmentation du calcium intracellulaire dans les cellules tubulaires rénales, induite par l'ionophore.

Hypercalcemia enhances tubular reabsorption of phosphorus by inhibiting endogenous secretion of parathyroid hormone [1,2], whereas restoration of serum calcium to normal in hypoparathyroidism causes phosphaturia by an unknown mechanism [3]. In addition, it has been proposed that hypercalcemia may act directly on the kidney to reduce urinary excretion of phosphorus, through several different mechanisms [2–9]. First, hypercalcemia may increase phosphate reabsorption by stimulating a parathyroid hormone-independent transport system in the kidney [5,6]. Second, hypercalcemia may suppress the phosphaturic effect of parathyroid hormone by inhibiting the hormone-induced activation of renal adenylate cyclase [7]. In a recent study, we were unable to demonstrate suppression of the phosphaturic action of parathyroid hormone by acute hypercalcemia in parathyroidectomized rats, even though i.v. calcium blunted the phosphaturic response to volume expansion [10]. These observations did not exclude the possibility that an increase in the intracellular calcium, as opposed to an increase in extracellular calcium, may interfere with the phosphaturic response to parathyroid hormone. The present study was designed to explore the latter possibility. To produce an increase in intracellular calcium, we employed a divalent cation ionophore which facilitates a passive entry of calcium into the cytoplasm and thus may suppress the activity of adenylate cyclase [11–14]. The ionophore does not disrupt the functional integrity of the cell membrane and does not interfere directly with

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the energy metabolism [13]. The results of the present study support the role of intracellular calcium in altering the renal response to parathyroid hormone.

Methods

White, female Sprague-Dawley rats (weighing 250 to 350 g) fed pellet chow diet (livestock and Poultry Feeds, Ralston Purina Co., St. Louis, MO) with tap water *ad lib* were studied.

Clearance studies. The clearance studies were performed in all animals at the same part of the day, between 8:00 A.M. and 4:00 P.M. After induction of anesthesia with an i.m. injection of sodium pentobarbital (40 mg/g of body wt), the animals were placed on heated operating boards and tracheostomy tubes were inserted. Rectal temperature was monitored by means of a thermistor and telethermometer (Yellow Springs Instrument Co., Yellow Springs, OH). The femoral artery and vein were exposed through inguinal incisions and PE-20 tubings (Clay Adams, Division of Becton, Dickinson, and Co., Parsippany, NJ) were inserted into each vessel. The arterial line was used for monitoring mean arterial blood pressure with a strain gauge (model 23AA, Statham Instruments, Inc., Oxnard, CA) and for the collection of blood samples. The venous line was extended to a syringe mounted on a variable speed continuous-infusion pump (Harvard Apparatus Co., Inc., Millis, MA). The urinary bladder was exposed and a funnel-shaped end of a PE-240 tubing was introduced through an incision at the fundus for urine collections. The incision was closed tightly with a purse string suture which excluded a major portion of the vesical lumen and thus eliminated most of the dead space. The technique of measuring inulin clearances (C_{In}) in rats and the analytical procedures used in our laboratory have been previously described in detail [15]. The sustaining infusion of inulin was given in 5% dextrose in water at the rate of 1.5 ml/100 g/hr. All plasma and urine specimens were analyzed for inulin [16] and phosphorus, and at least two plasma specimens obtained at the beginning and the end of each experiment were analyzed for calcium. Inorganic phosphorus was measured utilizing the methodology developed by the American Monitor Co. (American Monitor Patent No. 3-547-586), which is an automated modification of the method originally described by Fiske and Subbarow [17]. Calcium was determined with an atomic absorption spectrophotometer (model 290, Perkin-Elmer Corp., Norwalk, CT). The fractional phosphorus clearance was determined by factoring the clearance of phosphorus (C_P) by the clearance of inulin (C_{In}). Urinary cyclic 3',5'-adenosine monophosphate (cAMP) was determined in duplicates by the protein binding assay described by Gilman [18]. Calcium specific ionophore A 23187 (Eli Lilly and Company, Indianapolis, IN) was initially dissolved in a small amount of dimethyl sulfoxide (DMSO); 5 mg of ionophore in 0.25 ml of DMSO was used as the stock solution. For each individual experiment, the necessary amount of ionophore was obtained from the stock solution and dissolved in plasma separated from blood of rats similar to the experimental animals. In control groups, DMSO in equal volume but without ionophore was added to the plasma. In all experiments, the ionophore was given i.v. at the rate of 0.20 mg/kg of body wt/hr in the volume of 1 ml of plasma per hr. In preliminary testing, this dose was found to be well tolerated by most of the animals that were studied. Administration of higher doses of ionophore produced frequently a fall in blood pressure with a decrease in glomerular filtration. Animals that exhibited a decrease in blood pressure or glomerular filtration rate in response to a smaller dose of ionophore and their controls were excluded from the study.

Experimental groups: Group 1. In ten rats with intact parathyroid glands, after three control collections (each urine collection, 30 min), i.v. ionophore was given for four consecutive collections. Ten rats treated in a similar fashion but not receiving ionophore served as control.

Group 2. Parathyroidectomy (PTX) was performed two days before the clearance studies. In 12 PTX rats, following three control collections, ionophore was given i.v. for four consecutive periods. Twelve PTX rats treated in a similar fashion but not receiving the ionophore served as control.

Group 3. In eight PTX rats, after two control collections, parathyroid extract (PTE) (Eli Lilly and Co., Indianapolis, IN) was started i.v. at the rate of 1 U/100 g/hr. PTE was dissolved in 5% dextrose in water and given at the rate of 1 ml/min. After two clearance periods with PTE, i.v. ionophore was added and given with PTE for another four consecutive collections. Eight PTX rats treated in a similar fashion but not receiving the ionophore served as control.

Group 4. In four PTX rats after two control collections, i.v. ionophore was begun. After two clearance periods with ionophore, i.v. PTE was begun at the rate of 1 U/100 g/hr and was given with ionophore for another two colelction periods. Four additional PTX rats treated in a similar fashion but not receiving ionophore served as control.

In all experiments, the results were analyzed by comparing the experimental with the corresponding

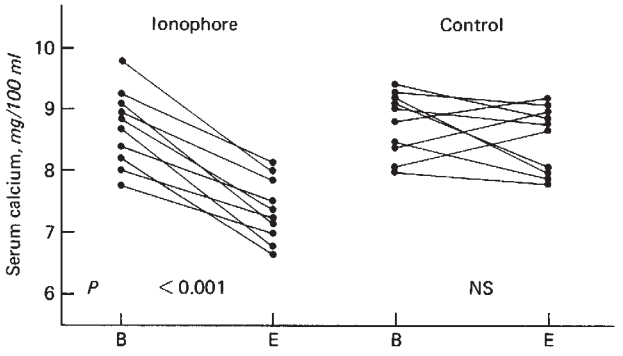

Fig. 1. *The effect of calcium ionophore on average serum calcium concentrations in intact rats before (B) and during (E) the administration of the ionophore in the experimental animals and administration of the vehicle in the control rats. P* refers to the difference between baseline and the experimental periods.

control groups, using the Student's *t* test. All results are presented in terms of mean ± SEM.

Results

Figure 1 depicts the effect of the ionophore on serum calcium concentrations in rats with intact parathyroid glands (group 1). Serum calcium decreased from a baseline of 8.7 ± 0.2 to an average of 7.5 ± 0.2 mg/100 ml ($P < 0.001$) during the administration of the ionophore. In the control animals, there was no change in serum calcium, and the corresponding concentrations were 8.7 ± 0.2 and 8.5 ± 0.2 mg/100 ml (P = NS). The average glomerular filtration rates for group 1 before and during the infusion of the ionophore are listed in Table 1. During all collection periods, there was no significant difference between the experimental and control animals. Likewise, there was no significant difference in serum phosphorus concentrations between the experimental and control animals.

Figure 2 depicts the alterations in fractional excretion of phosphorus to inulin (C_P/C_{In}) in group 1. During the baseline collections (0 to 90 min), there was no difference between the experimental and control animals. During the administration of the ionophore to the experimental animals (90 to 210 min), C_P/C_{In} in the control animals increased to 0.110 ± 0.020. In the experimental animals, C_P/C_{In} decreased to 0.019 ± 0.006 during the administration of the ionophore, resulting in a highly significant discrepancy between the control and experimental animals. Figure 3 shows the alterations in urinary excretion of cAMP following the administration of the ionophore. The data represent average values obtained by measuring cAMP in aliquots of pooled urine collections, both for the baseline (0 to 90 min) and the experimental periods (90 to 210 min). There was no difference in urinary excretion of cAMP between the control (119 ± 26 pmoles/min) and the experimental animals (158 ± 20) during the baseline collections. During the administration of the ionophore, urinary cAMP in the experimental animals decreased to 46 ± 16 pmoles/min and remained unchanged in the control animals, 131 ± 23 pmoles/min, resulting in a highly significant discrepancy between the control and the experimental animals. There was no significant difference between the groups in urinary excretion of calcium throughout all collection periods.

Figure 4 illustrates the effect of the ionophore on serum calcium concentrations in chronically PTX rats (group 2). In the experimental animals, serum calcium decreased from a baseline concentration of 6.5 ± 0.2, to 5.7 ± 0.2 mg/100 ml ($P<0.001$), whereas in the control animals the corresponding calcium concentrations were 6.5 ± 0.3 and 6.6 mg/100 ml (P=NS). Table 1 presents the glomerular filtration rates before and during the administration of the ionophore in group 2. During all collection periods, there was no difference between the control and experimental animals. Likewise, there was no difference in serum phosphorus concentrations and

Table 1. Inulin clearances during all collection periods in all groups of animals[a]

	0 to 30 min.	30 to 60 min.	60 to 90 min.	90 to 120 min.	120 to 150 min.	150 to 180 min.	180 to 210 min.	210 to 240 min.
Group 1								
Exp. (N = 10)	3.1 ± 0.4	3.8 ± 0.4	3.4 ± 0.6	3.1 ± 0.5	3.0 ± 0.4	2.8 ± 0.4	3.2 ± 0.4	—
Control (N = 10)	3.6 ± 0.4	3.5 ± 0.3	2.7 ± 0.3	3.9 ± 0.2	3.6 ± 0.2	2.6 ± 0.5	3.3 ± 0.3	—
Group 2								
Exp. (N = 12)	3.0 ± 0.3	3.3 ± 0.3	3.4 ± 0.3	3.5 ± 0.3	3.4 ± 0.2	3.2 ± 0.3	3.2 ± 0.4	—
Control (N = 12)	3.2 ± 0.3	3.3 ± 0.3	3.4 ± 0.2	3.5 ± 0.2	3.7 ± 0.2	3.6 ± 0.2	3.6 ± 0.2	—
Group 3								
Exp. (N = 7)	3.9 ± 0.3	3.6 ± 0.3	3.0 ± 0.3	4.0 ± 0.4	3.1 ± 0.3	3.1 ± 0.4	3.2 ± 0.3	3.4 ± 0.3
Control (N = 7)	3.6 ± 0.3	4.2 ± 0.6	3.9 ± 0.6	3.9 ± 0.3	3.8 ± 0.2	3.7 ± 0.3	4.0 ± 0.4	3.2 ± 0.2

[a] Exp. denotes experimental group. N = number of animals used. All values are ± SEM.

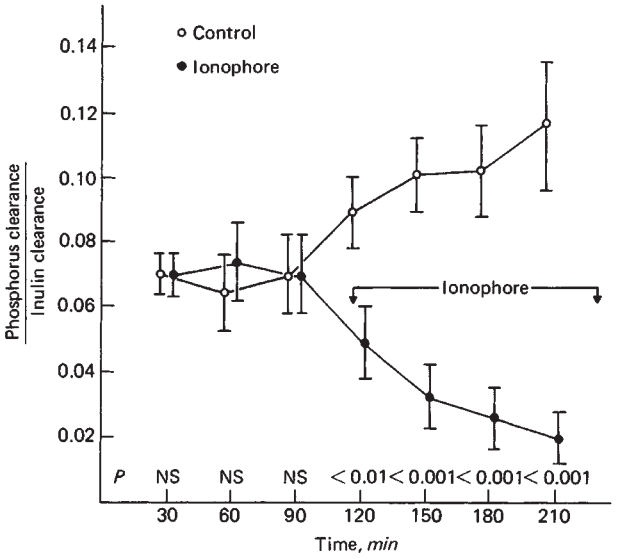

Fig. 2. *The effect of calcium ionophore on renal handling of phosphorus in intact rats. P* refers to the difference between the control and the experimental rats.

urinary excretion of calcium and cAMP. Figure 5 shows the variations in C_P/C_{In} in group 2. There was no difference between the experimental and control animals before (0 to 90 min) and during the administration of the ionophore (90 to 210 min).

Figure 6 depicts the alterations in C_P/C_{In} in group 3. During the baseline collections (0 to 60 min) and during the administration of parathyroid hormone (60 to 120 min), there was no significant difference between the control and experimental animals. During the combined administration of parathyroid hormone and ionophore (120 to 240 min), C_P/C_{In} in the experimental animals diminished to a nadir of 0.19 ± 0.04, whereas in the control animals that received parathyroid hormone, only C_P/C_{In} continued to increase, reaching a peak value of 0.43 ± 0.05. This resulted in a highly significant difference between the control and experimental animals. These changes were not associated with significant alterations in serum phosphorus or glomerular filtration rates (Table 1). Figure 7 illustrates the variations in urinary excretion of cAMP in group 3. During the baseline collections (0 to 60 min), there was no difference between the experimental, 34 ± 18 pmoles/min, and the control animals, 55 ± 20 pmoles/min (P = NS). During the administration of parathyroid hormone (60 to 120 min), urinary cAMP increased equally in the control, 281 ± 43 pmoles/min, and the experimental animals, 289 ± 23 pmoles/min. During the administration of the ionophore, however, urinary cAMP decreased to 159 ± 11 pmoles/min in the experimental animals and remained unchanged in the control animals, 254 ± 20 pmoles/min, resulting

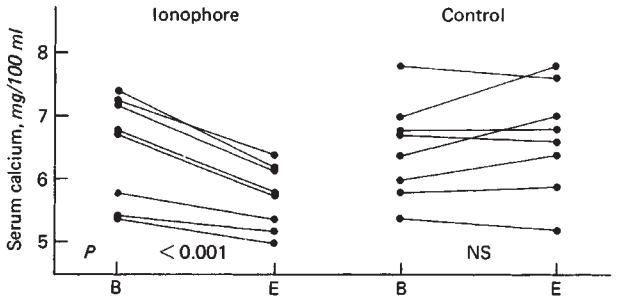

Fig. 4. *The effect of calcium ionophore on average serum calcium concentrations in parathyroidectomized rats, before (B) and during (E) the infusion of ionophore in the experimental animals and the infusion of the vehicle in the control rats. P* refers to the difference between the baseline and experimental periods.

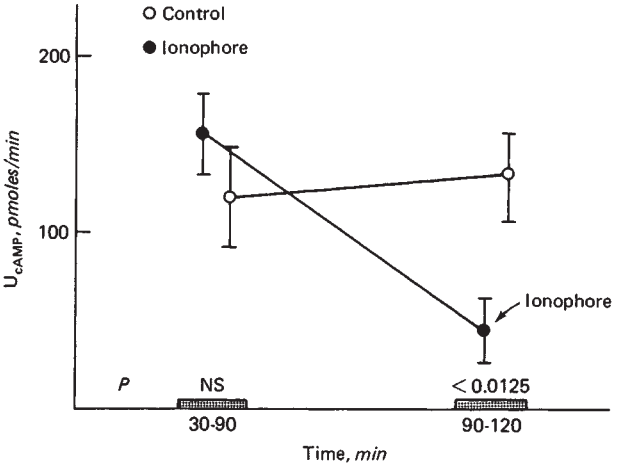

Fig. 3. *The effect of calcium ionophore on urinary excretion of cAMP.* The symbols represent excretion rates derived from pooled urine collections. *P* refers to the difference between the experimental and control animals.

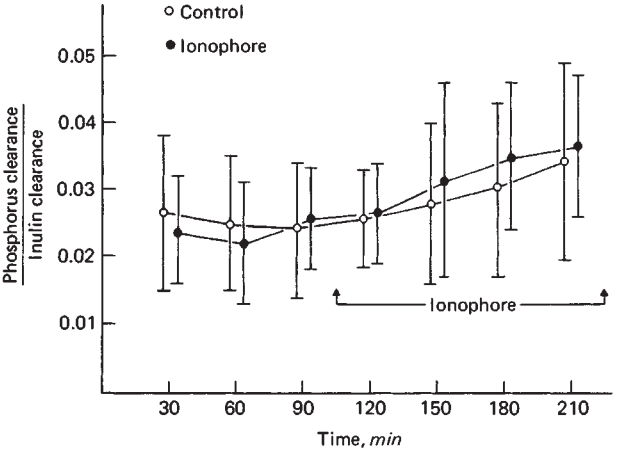

Fig. 5. *The effect of calcium ionophore on renal handling of phosphorus in parathyroidectomized rats.* There was no significant difference between the control and the experimental animals during all collection periods.

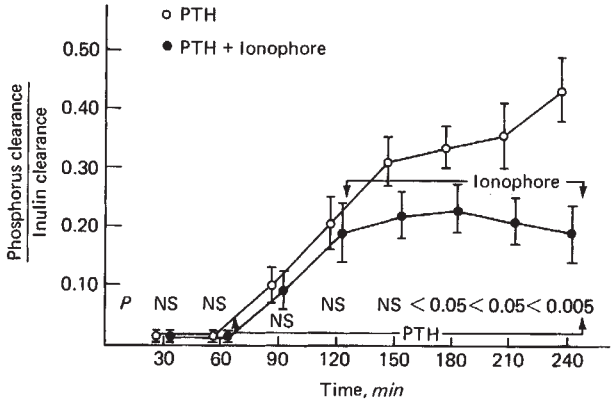

Fig. 6. *The effect of calcium ionophore on renal handling of phosphorus in parathyroidectomized rats receiving i.v. parathyroid hormone. P* refers to the difference between the control and experimental animals.

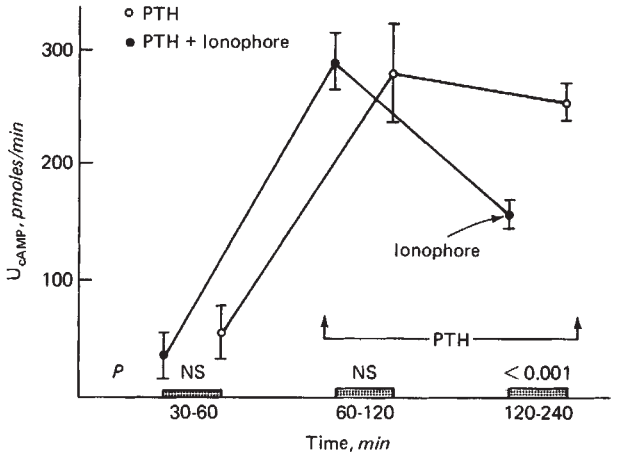

Fig. 7. *The effect of calcium ionophore on urinary excretion of cAMP in parathyroidectomized rats receiving i.v. parathyroid hormone (PTH).* The symbols represent excretion rates derived from pooled urine collections. *P* refers to the difference between the control and the experimental animals.

in a significant discrepancy in urinary excretion of cAMP between these two subgroups. There were no significant differences in serum concentrations or urinary excretion rates of calcium. The average serum calcium concentration decreased from a baseline of 7.1 ± 0.3 to an experimental level of 6.5 ±0.3 mg/100 ml; this fall, however, was not significant.

Table 2 shows the changes in serum concentrations of calcium and phosphorus, inulin clearances, and fractional excretion of phosphorus in group 4. In the experimental animals, serum calcium concentration (S_{Ca}), during the combined infusion of ionophore and PTH (S_{Ca}, 5.1 ± 0.3), was lower than that in the control animals (6.5 ± 0.3, $P<0.01$) and also lower than that recorded in the same animals during the baseline clearances (6.7 ± 0.3 mg/100 ml, $P<0.01$). During PTH infusion, the C_P/C_{In} in the control animals (0.109 ± 0.009) was lower than the C_P/C_{In} in the experimental animals (0.044 ± 0.012, $P<0.005$) that were receiving a combination of ionophore and PTH.

Discussion

The present study has demonstrated that calcium ionophore reduces urinary excretion of phosphorus

Table 2. Average serum concentrations of calcium (S_{Ca}) and phosphorus (S_P), inulin clearances (C_{In}) and fractional excretion of phosphorus (C_P/C_{In}) in four experimental parathyroidectomized (PTX) rats during two baseline periods (0 to 60 min), two ionophore periods (60 to 120 min) and two ionophore + parathyroid hormone (PTH) periods (120 to 180 min) compared with corresponding values in four control PTX rats not receiving ionophore.

	Baseline				Ionophore				Ionophore + PTH			
	C_{In} ml/min	$\frac{C_P}{C_{In}}$	S_{Ca} mg/100 ml	S_P mg/100 ml	C_{In} ml/min	$\frac{C_P}{C_{In}}$	S_{Ca} mg/100 ml	S_P mg/100 ml	C_{In} ml/min	$\frac{C_P}{C_{In}}$	S_{Ca} mg/100 ml	S_P mg/100 ml
Experimental rats												
1	2.50	0.050	7.0	6.5	2.10	0.055	6.2	7.0	1.60	0.012	5.1	6.4
2	2.45	0.015	7.1	4.5	2.32	0.015	6.9	4.4	2.70	0.045	6.0	5.1
3	3.40	0.045	6.9	7.1	3.65	0.035	6.1	6.5	2.22	0.075	5.0	5.4
4	3.60	0.012	5.9	7.8	3.60	0.056	5.9	6.4	3.82	0.045	4.5	7.0
Mean	2.99	0.030	6.7	6.5	2.92	0.040	6.2	6.1	2.58	0.044	5.1	6.0
± SEM	0.29	0.009	0.3	0.7	0.41	0.009	0.2	0.6	0.47	0.012	0.3	0.4
Control rats												
1	3.85	0.023	7.1	6.4	3.95	0.019	6.9	6.5	3.95	0.136	6.9	5.4
2	3.98	0.002	5.9	6.0	3.33	0.002	5.6	5.4	3.18	0.106	5.7	5.1
3	2.50	0.065	6.5	7.5	2.51	0.056	6.6	7.1	2.15	0.101	6.6	7.2
4	1.81	0.048	7.4	5.4	1.75	0.042	7.0	5.5	1.65	0.095	7.0	5.9
Mean	3.03	0.034	6.7	6.3	2.88	0.030	6.5	6.1	2.73	0.109	6.5	5.9
± SEM	0.52	0.013	0.3	0.4	0.48	0.011	0.3	0.4	0.52	0.009	0.3	0.5
P[a]	NS	NS	NS	NS	NS	NS	NS	NS	NS	<0.005	<0.01	NS

[a] Relates to the difference between the experimental and control animals.

in rats. Since no consistent changes in the filtered load were noted, if may be concluded that the ionophore produced a net increase in tubular reabsorption of phosphorus. The associated decrease in serum calcium concentration, in absence of urinary losses, is consistent with a shift of calcium from the extracellular to the intracellular compartment. It is therefore likely that the ionophore exerts its effect on calcium distribution, not only *in vitro* but also in intact animals, without compromising the vital functions, at least at the dose used by us. The lack of significant drop in serum calcium in group 3, as opposed to its occurrence in group 4 is not well understood. It is possible that the prolonged administration of PTH could offset the hypocalcemic effect of ionophore in group 3, whereas with the relatively longer administration of ionophore, a shorter administration of PTH in group 4 led to significant hypocalcemia.

The cytoplasmic calcium concentration is maintained very low as a result of an active process which extrudes the calcium from the cytosol at a rate which exceeds the passive back-diffusion down a chemical gradient. It is believed that the active energy-dependent transport system operates both at the cell membrane and at the mitochondrial membrane, which accounts for the high intramitochondrial calcium content [19,20]. Calcium ionophore (A 23187) can increase the intracytoplasmic calcium by facilitating the passive back diffusion to an extent that it will exceed the active extrusion [11-13, 21,22]. The ionophore catalyzes transport by *1)* enveloping an ion at a membrane interphase with a consequent dehydration of the ion, *2)* diffusing across the membrane as a cation complex, *3)* releasing the ion which undergoes concomitant rehydration at the opposite interphase, and *4)* diffusing back uncomplexed to the original interphase to complete the catalytic process [12]. The ionophore also enters mitochondrial membranes and acts as a freely mobile carrier to equilibrate divalent ion concentrations across the membranes [11]. The ionophore, however, does not disrupt the functional integrity of the membrane and does not interfere directly with the energy metabolism [22]. Therefore, ionophore plus calcium may serve as an experimental by-pass of hormone receptors in intact cells.

The mechanism(s) by which the ionophore enhances tubular reabsorption of phosphorus is not well defined by the present findings; several possibilities, however, are worth comment. First, it could act directly on the kidney to augment phosphate transfer through a parathyroid hormone independent system. This action could be mediated through alterations in intrarenal hemodynamics, and/or through a direct effect on tubular transport. The lack of an antiphosphaturic response in parathyroidectomized animals militates against this possibility and implicates the presence of circulating parthyroid hormone as an essential factor in the effect of the ionophore. It has to be realized, however, that the low baseline C_P/C_{In} in group 2 was an unfavorable condition for the testing of the effect of ionophore to enhance tubular reabsorption of phosphorus.

Second, the ionophore could act indirectly on tubular transfer of phosphorus by suppressing secretion of parathyroid hormone by the parathyroid glands. This action could be mediated either *a)* by increasing the intracytoplasmic calcium and/or *b)* blocking the β-adrenergic tone in the parathyroid glands [23]. The latter effect has been also demonstrated in other biologic systems [14]. The presence of an antiphosphaturic effect of ionophore in parathyroidectomized rats infused with parathyroid hormone suggests that the presence of parathyroid tissue is not absolutely necessary for this effect, even though the ionophore may alter its secretory activity. Third, the ionophore could act on the tubular transfer of phosphorus by blocking the phosphaturic effect of parathyroid hormone. The observations made in parathyroidectomized rats receiving parathyroid hormone infusion, in which the ionophore blocked the phosphaturic response to the hormone, is consistent with this mechanism of action. There is experimental evidence suggesting that increased intracytoplasmic calcium blocks the parathyroid hormone–induced activation of renal adenylate cyclase [24,25]. *In vitro* studies showed that renal cortical adenyl cyclase activity could be blocked by calcium at concentrations of 3 mM in homogenates of tissue [25]. When similar concentration of calcium was present in media containing intact tubular cells, however, it did not have an appreciable effect on the activity of adenyl cyclase [26]. Theoretically, this observation suggests that the intracellular but not the extracellular calcium concentration is responsible for changes in adenyl cyclase activity. It has been demonstrated *in vitro* that the ionophore, by an increase in intracellular calcium, may inhibit the activation of adenylate cyclase [14]. It is possible that a similar reaction may occur *in vivo* and be responsible for the observed alterations in renal handling of phosphorus. Thus, the ionophore, by increasing intracytoplasmic calcium, could inhibit the parathyroid hormone-induced activation of renal adenylate cyclase and reduce the urinary excretion of phosphorus. The observed changes in the urinary excretion of cAMP, both in intact and parathyroid hormone-infused parathyroidectomized rats, lend support to this contention. The possibility that the

movement of magnesium down its chemical gradient might be responsible for the observed changes cannot be ruled out on the basis of the data available.

In the present study, renal content of cAMP was not measured. Thus, it has not been established with certainty that the urinary changes reflected changes in renal formation of cAMP. These changes might represent altered cellular permeability to cAMP. Furthermore ionophore might act on extrarenal organs to suppress the release of cAMP and, thus, reduce its urinary excretion. Almost all urinary cAMP in the rat is derived from the nephrogenously generated cyclic nucleotide, most of which is under the control of parathyroid hormone [27]. Suppression of secretion of PTH in the rat causes a marked reduction in the nephrogeneous cAMP contribution, which accounts for most of cAMP excreted in the urine by the rat [27]. It is, therefore, most likely but not certain that the observed changes in urinary cAMP in this study represented changes in the nephrogenously generated nucleotide.

The present findings do not rule out other cAMP indpendent mechanisms by which increased intracytoplasmic calcium could blunt the phosphaturic action of parathyroid hormone. It is also possible that an increase in intracellular calcium may stimulate the activity of guanalyl cyclase and thus increase the generation of cyclic guanosine monophosphate (cGMP). The role of cGMP, however, in the phosphaturic response to PTH has not been established yet [28].

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