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Colchicine blocks the action of parathyroid hormone but not nicotinamide on renal phosphate transport

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Nicotinamide, like parathyroid hormone, is a rapidly acting specific inhibitor of Na^+-dependent transport of phosphate (P_i) across the brush-border membrane of the proximal tubule of the mammalian kidney. Pretreatment of rats with colchicine (0.7 mg/kg body weight) for 1 h led to a significantly diminished phosphaturic response to parathyroid hormone (synthetic 1–34 fragment, 4 μg/kg). In contrast, the same dose of colchicine had no effect on the renal response to nicotinamide (1.0 g/kg), measured both as the change in urinary P_i excretion and as Na^+-dependent P_i uptake by isolated brush-border membrane vesicles. These data suggest indirectly that the intracellular mechanism that mediates the inhibitory effects of nicotinamide on renal P_i transport does not require intact microtubules.

Introduction

Nicotinamide acts on the renal proximal tubule [1] to specifically inhibit reabsorption of inorganic phosphate (P_i). The decrease in P_i reabsorption is accompanied by an increase in NAD content of the renal cortex, by specific inhibition of the Na^+-dependent P_i transport system in the luminal brush-border membrane of the proximal tubule, and by an increase in the urinary excretion of P_i [2]. The changes in P_i transport may be mediated in part by interaction of cytosolic NAD with the P_i transport system in the brush-border membrane. This interaction may involve ADP-ribosylation of specific membrane proteins that are either part of or are closely associated with the P_i transporter [3]. Recent studies with 5-methylnicotinamide, which does not change the NAD level in renal cortex [4], indicate that the action of nicotinamide may not be mediated solely by the increase in NAD.

Nicotinamide may be classified as a rapidly acting inhibitor of the brush-border P_i transport system [5] based on the observations that the onset of its action is detectable within 1 h [6] and its inhibitory effect does not require de novo protein synthesis [7]. Parathyroid hormone (PTH) is a physiological regulator of the brush-border transport system for P_i and, like nicotinamide, this hormone acts rapidly. Both PTH and nicotinamide produce a decrease in the P_i transport capacity (V_{max}) without affecting the apparent affinity (K_m) [5].

The inhibitory action of PTH is blocked when

Abbreviations: P_i, inorganic phosphate; PTH, parathyroid hormone; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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rats are injected with colchicine, a microtubule disrupting agent, prior to administration of the hormone [8]. In contrast, rats treated with lumicolchicine, an analog of colchicine that does not interfere with microtubular structure, show a normal phosphaturic response to PTH [8]. These findings indicate that the intracellular mechanism of action of PTH requires the presence of intact microtubules, and lend support to the idea that rapidly acting regulators of the renal P_i transport system may exert their effects by altering the recycling of P_i transporters between the luminal brush-border membrane and a cytosolic pool [5,9], a process that is likely to involve the cell cytoskeleton.

Since the final effect of nicotinamide on the renal brush-border P_i transporter is the same as the effect of PTH, it is possible that the intracellular mechanism of action of these phosphaturic agents may converge in a final common pathway. The present study was designed to determine if the phosphaturic action of nicotinamide, like PTH, is disrupted by colchicine.

Methods

Male Sprague-Dawley rats weighing 140–150 g were used in all experiments and were housed individually in metabolic cages. In the experiments with PTH the rats were fed normal P_i diet (Ralston Purina Co., St. Louis, MO) containing 0.86% P_i. In the experiments with nicotinamide a different group was fed low-P_i diet containing 0.1% P_i (Bioserve Inc., Frenchtown, NJ) for four days in order to establish a maximal rate of renal P_i reabsorption and a low baseline for urinary P_i excretion [4]. Synthetic bovine PTH 1–34 fragment (activity 8000 U/mg, Sigma Chemical Co., St. Louis, MO), nicotinamide, colchicine and lumicolchicine were dissolved in saline and each drug was given as a single injection. PTH was given intravenously at a dose of 4 μg/kg body weight [1]. The dose of nicotinamide (1.0 g/kg) was chosen to achieve maximum inhibition of renal P_i transport, based on previous observations [2]. The dose of colchicine (0.7 mg/kg) was determined in preliminary experiments, and was similar to that used previously to disrupt microtubules in vivo in rats [10–13]. It is within the range that decreases tissue microtubule content [12], and it is greater than the dose that blocks the antidiuretic effect of vasopressin [11]. Rats pretreated with colchicine, or the same dose of lumicolchicine, received these drugs by intraperitoneal injection 1 h before administration of either PTH or nicotinamide. Control rats were injected with an equal volume of saline. Urine was collected for the next 8 h, after which time the rats were anesthetised with ether, blood samples were obtained from the abdominal aorta, and the kidneys were removed. The different groups of rats in each experimental series (see Results) were processed in parallel in every experiment.

Brush-border membrane vesicles were prepared from homogenised renal cortex by divalent cation precipitation using two $MgCl_2$ treatments as described in detail elsewhere [14]. The purity of the final membrane fraction was determined routinely by assaying the activity of alkaline phosphatase. The activity of this enzyme was enriched 8-fold in the brush-border membrane fraction compared to the starting homogenate. The procedure for measuring Na^+-gradient-dependent P_i uptake by isolated membrane vesicles was identical to that described previously in detail [2,4]. Briefly, 15 μl of a vesicle suspension in 300 mM mannitol, 5 mM

TABLE I

EFFECT OF COLCHICINE AND LUMICOLCHICINE ON THE PHOSPHATURIC RESPONSE TO PARATHYROID HORMONE IN RATS FED NORMAL P_i DIET

Values are means ± S.E. The number of rats in each group is given in parentheses. * indicates significantly different ($P < 0.05$, group t-test) compared to the value in rats given PTH alone.

Treatment		Urinary P_i excretion (μmol P_i/mg creatinine)	
		before injection	after injection
Experiment 1			
Colchicine	(4)	8.0 ± 2.5	9.2 ± 2.6 *
PTH	(3)	7.5 ± 4.5	71.4 ± 6.7
Colchicine + PTH	(3)	10.8 ± 7.5	38.9 ± 9.3 *
Experiment 2			
Lumicolchicine	(4)	7.5 ± 4.1	20.6 ± 4.2 *
PTH	(4)	8.0 ± 1.3	51.3 ± 11.0
Lumicolchicine + PTH	(4)	8.6 ± 2.6	61.0 ± 21.1

Tris (pH 7.4 with Hepes) was added to 30 μl of incubation medium containing (final concentrations) 100 mM NaCl, 100 mM mannitol, 0.1 mM KH_2 $^{32}PO_4$, 5 mM Tris (pH 7.4 with Hepes). Uptake was stopped after either 10 s (initial phase of uptake) or 100 min (equilibrium point) [2,4] by addition of 2.0 ml of ice-cold stopping solution containing 145 mM NaCl, 10 mM sodium arsenate, 5 mM Tris-HCl (pH 7.4). The membrane vesicles were recovered by Millipore filtration, washed and processed for liquid scintillation counting. All data were corrected for binding of radioactivity to the membranes and filters by running the appropriate blanks [2,4].

Creatinine and P_i in deproteinised plasma and urine, and protein and enzyme activities in the membrane fractions and homogenates were assayed by the procedures described previously in detail [2,4].

Results and Discussion

It was shown previously that colchicine alone, given as two injections each of 2.2 mg/kg body weight, increased the fractional excretion of P_i [8]. The initial experiments in the present study used rats fed normal P_i diet to determine the dose of colchicine that did not change the renal excretion of P_i, when given alone, but was sufficient to interfere with the phosphatic response to PTH. These requirements were met by a single intraperitoneal injection of colchicine at a dose of 0.7 mg/kg given 1 h prior to administration of PTH. At this dose, colchicine alone produced no significant change in urinary P_i excretion, expressed relative to endogenous creatinine excretion, but the phosphaturic response to PTH was blunted (Table I, Expt. 1). PTH alone produced a 10-fold increase ($P < 0.02$, paired t-test) in urinary excretion of P_i compared to the level immediately prior to injection of PTH. This effect of PTH was significantly decreased in rats pretreated with colchicine. Urinary P_i excretion in these rats was increased only 3.6-fold by PTH and was not significantly different ($P > 0.05$, paired t-test) from the P_i excretion prior to drug treatment.

The same dose of lumicolchicine, an inactive analog, did not interfere with the phosphaturic response to PTH (Table I, Expt. 2). Again, PTH alone caused a large increase in urinary P_i excretion compared to the level before injection ($P < 0.01$, paired t-test). This action of PTH was undiminished in the rats pretreated with lumicolchicine, urine P_i excretion increased 7.1-fold over the value prior to drug treatment ($P < 0.01$, paired t-test). These findings strongly suggest that the effects of colchicine are the result of its microtubule disrupting action.

After establishing the dose of colchicine that was effective in blunting the phosphaturic action of PTH, the next series of experiments used rats adapted to low-P_i diet to determine if the same dose of colchicine also interfered with the action of nicotinamide on renal P_i transport.

Clearance of endogenous creating in the drug-treated groups was not different from controls, indicating that neither colchicine nor nicotinamide produced major changes in the glomerular filtration rate (Table II).

Prior to drug treatment, there were no significant differences in urinary P_i excretion between the four groups of rats (data not shown). Colchicine alone did not affect urinary excretion of P_i or the plasma level of P_i (Table II). As expected, nicotinamide alone produced a large (30-fold) increase in urinary P_i excretion compared to control

TABLE II

CREATININE CLEARANCE (C_{cr}), PLASMA PHOSPHATE (P_i), AND URINARY P_i EXCRETION IN RATS FED LOW-P_i DIET

The dose of colchicine was 0.7 mg/kg body weight, and the dose of nicotinamide was 1.0 g/kg body weight. Each drug was administered as a single intraperitoneal injection. When both drugs were administered, the colchicine was given first and the nicotinamide was given 1 h later. All rats were killed 8 h after the injections. Values are the means ± S.E. of five rats in each group. * indicates significantly different ($P < 0.05$, group t-test) compared to the group treated with the saline vehicle.

Treatment	C_{cr} (ml/24 h per 100 g body wt.)	Plasma P_i (mM)	Urine P_i (μmol P_i/mg creatinine)
Saline vehicle	484 ± 65	1.69 ± 0.21	0.16 ± 0.15
Colchicine	451 ± 71	1.94 ± 0.15	0.04 ± 0.01
Nicotinamide	431 ± 26	2.76 ± 0.07 *	4.87 ± 1.45 *
Colchicine + nicotinamide	441 ± 54	2.96 ± 0.23 *	10.58 ± 3.70 *

rats given the saline vehicle. This is a specific effect on P_i transport because previous studies have shown that the urinary excretion of other ions is not increased by nicotinamide [2]. Although plasma P_i was increased in the nicotinamide treated group (Table II) it is unlikely that this change is the cause of the increased urinary excretion of P_i in these rats. A similar increase in plasma P_i induced by cycloheximide, in a separate study, did not change urinary P_i excretion in low-P_i-diet rats [7]. Furthermore, renal P_i excretion in low-P_i-diet rats is not increased by many stimuli that are phosphaturic in rats fed normal P_i diet [4]. The phosphaturic action of nicotinamide is observed also in thyroparathyroidectomised rats [2] indicating that it is not mediated by PTH.

In the group of rats pretreated with colchicine for 1 h prior to administration of nicotinamide, the colchicine did not prevent the increase in urinary excretion of P_i (Table II). If anything, the increase in P_i excretion compared to the controls (66-fold) tended to be greater than the increase due to nicotinamide alone.

In order to determine if colchicine interfered with with nicotinamide action at the level of the brush border P_i transporter, the Na^+-gradient-dependent uptake of P_i by brush-border membrane vesicles isolated from the renal cortices of the same groups of rats was assessed (Table III). The uptake of P_i by vesicles from saline-treated rats was 3-fold greater at 10 s, the initial part of the overshoot phase of uptake, compared to the uptake at 100 min, the equilibrium phase of uptake [2,4]. Colchicine alone did not change P_i transport either at 10 s or 100 min compared to the controls (saline-treated). The results of other experiments (not shown) indicate that this dose of colchicine also did not affect the Na^+-gradient-dependent transport of other solutes such as proline.

Nicotinamide alone, as expected, produced significant inhibition of P_i transport at 10 s, the uptake was decreased by 40% compared to controls. The 100 min uptake was not different from the controls indicating that the decrease in the 10 s uptake was not due to a difference in vesicle size. The inhibitory action of nicotinamide on brush-border membrane transport of P_i is a specific one. It has been shown previously that only the Na^+-dependent component of P_i transport is altered, the inhibition is not due to dissipation of the Na^+ gradient, and other Na^+-gradient-dependent transport systems remain unaltered [2]. Furthermore, nicotinamide itself has no direct action on P_i transport when added to isolated brush-border membrane vesicles [2].

The 10 s P_i uptake by vesicles from the rats treated with colchicine prior to nicotinamide injection also was significantly inhibited compared to saline-treated controls. The degree of inhibition (42%) was comparable to that achieved by nicotinamide alone (Table III). The 100 min P_i uptake in this group, as with the other groups, was not different from the uptake in the control group. These data indicate that the inhibitory effect of nicotinamide on the P_i transporter in the renal brush-border membrane was not blocked by pretreatment of rats with colchicine.

In conclusion, renal transport of P_i was determined both indirectly as urinary P_i excretion, and directly as Na^+-gradient-dependent P_i uptake by isolated brush-border vesicles. The results of both of these procedures indicated that the inhibitory action of nicotinamide on renal P_i transport was not disrupted by colchicine. These findings indicate, indirectly, that intact microtubules may not be involved in the intracellular mechanism that mediates the action of nicotinamide in the renal proximal tubule. In this regard, the action of

TABLE III

EFFECT OF COLCHICINE AND NICOTINAMIDE ON Na^+-DEPENDENT TRANSPORT OF P_i BY RENAL BRUSH-BORDER MEMBRANE VESICLES FROM RATS FED LOW-P_i DIET.

The uptake of P_i was determined both during the initial uphill phase (10 s) and at the equilibrium point (100 min). Values are the means ± S.E. of five separate membrane preparations from each group. * indicates significantly different ($P < 0.05$, group t-test) compared to the uptake in vesicles from rats given saline vehicle. See Table II for other details.

Treatment	Na^+-dependent P_i uptake (pmol/mg membrane protein)	
	10 s	100 min
Saline vehicle	613 ± 41	199 ± 18
Colchicine	501 ± 66	199 ± 20
Nicotinamide	375 ± 59 *	234 ± 48
Colchicine + nicotinamide	362 ± 84 *	186 ± 28

nicotinamide differs from the colchicine-sensitive action of PTH, even though both agents can be classified as rapidly acting and both agents have the same final effect on the P_i transporter in the luminal brush-border membrane [5].

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