Locally formed 5-hydroxytryptamine stimulates phosphate transport in cultured opossum kidney cells and in rat kidney

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Renal proximal tubular cells have been shown to express aromatic L-amino acid decarboxylase (L-AAAD), which converts L-dopa into dopamine and 5-hydroxytryptophan [(OH)Trp] into 5-hydroxytryptamine (5-HT; serotonin). Because 5-HT receptors have been demonstrated in proximal cells, we hypothesized that 5-HT may act as an autocrine/paracrine modulator of proximal transport. We evaluated this possibility in opossum kidney (OK) cells, a renal epithelial cell line with a proximal phenotype expressing 5-HT_{1B} receptors, and in intact anaesthetized rats. 5-HT synthesis by OK cells increased with incubation time and (OH)Trp concentration, and was abolished by benserazide, an L-AAAD inhibitor. 5-HT reversed parathyroid hormone (PTH)-induced cAMP accumulation in a pertussis toxin-sensitive

manner and reduced the PTH inhibition of P_i uptake without affecting the NaP_i-4 mRNA level. The effects of 5-HT on cAMP generation and Na–P_i co-transport were reproduced by (OH)Trp, except in the presence of benserazide, and by L-propranolol and dihydroergotamine, two 5-HT_{1B} receptor agonists. In rats, (OH)Trp and dihydroergotamine decreased fractional P_i excretion. Benserazide abolished the effect of (OH)Trp but not that of dihydroergotamine. In conclusion: (i) locally generated 5-HT blunts the inhibitory effect of PTH on Na–P_i co-transport in OK cells; (ii) endogenous 5-HT decreases P_i excretion in rats; and (iii) 5-HT is a paracrine modulator involved in the physiological regulation of renal P_i transport.

INTRODUCTION

The initial and limiting step of P_i reabsorption by the renal proximal tubule, i.e. P_i entry into tubular cells from the tubular lumen, takes place via the sodium–phosphate (Na–P_i) co-transporter located in renal brush-border membranes. This transport system has been identified as a crucial target of dietary, endocrine and autocrine/paracrine factors acknowledged as modulators of renal P_i handling (for reviews, see [1,2]). Na–P_i co-transport activity is increased by a low-phosphate diet, thyroid hormone and growth hormone, whereas it is decreased by parathyroid hormone (PTH) [1,2]. The recent cloning of renal Na–P_i co-transporter isoforms in several species, including the human, has provided new and important tools allowing analysis of the regulation of renal P_i transport at a molecular level [3].

The bioactive amines 5-hydroxytryptamine (5-HT; serotonin) and dopamine have been reported to be local modulators of tubular transport [4–19]. 5-HT and dopamine share several features: (i) they are synthesized by proximal tubular cells, from tryptophan and L-dopa respectively [6–8,10–12,17,18,20]; (ii) the same enzyme, aromatic L-amino acid decarboxylase (L-AAAD), is involved in their synthesis [18–20]; and (iii) both serotonergic and dopaminergic receptors have been detected in the kidney [5,11,17,21–24]. While the phosphaturic effect of dopamine has been abundantly documented by *in vitro* and *in vivo* studies [6,8,10], an effect of 5-HT on renal P_i handling has not been reported. However, the possibility that 5-HT might affect Na–P_i co-transport is suggested by data obtained with opossum kidney (OK) cells, an epithelial cell line with a proximal tubular phenotype in which Na–P_i co-transport is regulated by the

cAMP/protein kinase A pathway [1–3]. In these cells 5-HT, through the occupancy of 5-HT_{1B} receptors negatively coupled to adenylate cyclase, blunts parathyroid hormone (PTH)-stimulated cAMP generation [21–24].

The aim of the present study was to test the hypothesis that 5-HT, locally generated by proximal cells, is a physiological modulator of Na–P_i co-transport. We show that OK cells synthesize 5-HT from its precursor and that 5-HT blunts the inhibition of Na–P_i co-transport activity by PTH. Moreover, an anti-phosphaturic effect of locally formed 5-HT was shown in the rat. These data demonstrate that 5-HT is a paracrine modulator of renal P_i transport.

Part of this work was presented at the 27th Annual Meeting of the American Society of Nephrology (Orlando, Florida, October 1994) and presented in abstract form [25].

MATERIALS AND METHODS

Materials

Benserazide was kindly donated by Hoffman–La Roche Laboratories. Tracers were from the following sources: K₂H³²PO₄ from New England Nuclear (Boston, MA, U.S.A.), methyl- α -D-[U-¹⁴C]glucopyranoside ([¹⁴C]MGP) and L-[2,3-³H]alanine from Amersham (Amersham, Bucks., U.K.). Culture media and reagents were from Techgen (Les Ulis, France). Plasticware was from Costar (Cambridge, MA, U.S.A.). The PTH peptide used in this study was the 1–34 fragment of bovine PTH. Other compounds were purchased from Sigma (St. Louis, MO, U.S.A.).

Abbreviations used: L-AAAD, aromatic L-amino acid decarboxylase; DHE, dihydroergotamine; 5-HT, 5-hydroxytryptamine (serotonin); MGP, methyl- α -D-glucopyranoside; (OH)Trp, 5-hydroxytryptophan; OK cells, opossum kidney cells; PTH, parathyroid hormone.

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Figure 1 5-HT synthesis by OK cells

(A) OK cells were incubated with 10 μ M (OH)Trp for 5–60 min. (B) OK cells were incubated for 60 min with (OH)Trp at the indicated concentrations in the absence (●) or presence (○) of 100 μ M benserazide. At the end of incubation, supernatants were acidified and stored at -20°C until measurement of 5-HT was performed as indicated in the Materials and methods section. Results are means $\pm$ S.E.M. of three different experiments in which duplicates were obtained.

Cell culture

OK cells (passages 80–100) were grown to confluence in 6-well or 24-well trays as previously described [25a]. Monolayers reached confluence after 4 days, and they were used for experiments 2 or 3 days after confluence was achieved. On the day prior to experiments, culture medium was changed to hormone-free and serum-free medium. Cells were subcultured weekly by trypsinization. The splitting ratio was 1:5.

Determination of 5-HT and dopamine accumulation

For determination of 5-HT synthesis by OK cells, cells grown in 6-well trays were incubated at 37°C for 30 or 60 min in 2 ml/well of a buffered solution with the following composition (mmol/l): 137 NaCl, 5.4 KCl, 1 CaCl_2 , 1.2 MgSO_4 , 1 NaHPO_4 , 15 Hepes, 5 glucose (pH 7.4). Various concentrations of 5-hydroxytryptophan [(OH)Trp] or benserazide were added to the medium. Stock solutions of these compounds were prepared in the same solution containing 1 mM ascorbic acid in order to prevent oxidation. At the end of incubation, 1.5 ml aliquots of the incubation medium were transferred to plastic tubes containing 10 μ l of 13 M HCl. Tubes were stored at -20°C until analysis. In *in vivo* studies, rat urine samples were collected into tubes containing 10 μ l of 13 M HCl. Tubes were stored at -20°C until analysis, as above. Determination of 5-HT and dopamine concentrations in cell supernatants and in urine was performed by HPLC and electro-detection [26–28].

Uptake studies

After incubation of cells with hormones and drugs, usually for 3 h [25a], uptake of P_i , MGP and alanine was measured as previously described [12,25a,29]. Briefly, uptake was measured at 37°C in a buffered solution with the following composition (mmol/l): 137 NaCl, 5.4 KCl, 1 CaCl_2 , 1.2 MgSO_4 , 15 Hepes (pH 7.4). The sodium-free solution was made iso-osmotic by replacing NaCl with *N*-methyl-D-glucamine. After removal of culture medium, cells were washed with 1 ml of uptake solution/well, and were then incubated for various periods of time in the presence of $\text{K}_2\text{H}^{32}\text{PO}_4$ (0.5 $\mu\text{Ci/ml}$), $[\text{14C}]\text{MGP}$ (0.5 $\mu\text{Ci/ml}$) or L-[2,3- ^3H]alanine (1 $\mu\text{Ci/ml}$) plus 100 μM KH_2PO_4 , 1 mM

MGP or 100 μM L-alanine respectively. All steps were performed at 37°C . At the end of incubation, uptake was stopped by washing the cells three times with 1 ml of ice-cold buffer/well (137 mM NaCl, 15 mM Hepes, pH 7.4). Cells were then solubilized in 0.5% Triton X-100 (250 $\mu\text{l/well}$) and aliquots were counted for radioactivity by liquid scintillation counting.

Determination of intracellular cAMP content

After removal of culture medium, cells were washed with 1 ml/well of Hank's balanced salt solution supplemented with 15 mM Hepes and 2 mM L-glutamine, and were then pre-incubated for 15 min at 37°C in the same buffer (pH 7.4) (500 $\mu\text{l/well}$) containing 1 mM ascorbic acid and 0.1 mM isobutylmethylxanthine, a phosphodiesterase inhibitor. Medium was then removed and cells were incubated, usually for 20 min, in a similar solution to which hormones were added. At the end of incubation, the intracellular cAMP content was measured by radioimmunoassay as previously described [25a].

RNA extraction and Northern blot analysis

Total RNA was extracted from OK cells by the guanidinium isothiocyanate method [30]. Samples of 20 μg of total RNA were separated through 1% agarose gels containing 6.7% formaldehyde and transferred to a nylon membrane. Hybridization was performed at 40°C for 20 h in $5 \times \text{SSPE}$, 40% formamide, $5 \times \text{Denhart's}$ solution and 100 $\mu\text{g/ml}$ salmon sperm DNA.

The probe NaP_i -4 was obtained by reverse transcription of OK cell RNA, and the cDNAs were amplified by PCR using the following primers: upper, 5'-CCTCACATCCATCATCGTC-AG-3'; lower, 5'-AGATAGAGGACGGCAAACCAG-3'. The DNA amplification product was 1042 bp long and was labelled by random priming. To measure levels of glyceraldehyde-3-phosphate dehydrogenase mRNA, blots were hybridized with a cloned rat cDNA for this protein.

In vivo studies

Experiments were performed using 14 male Sprague–Dawley rats of average body weight 198 ± 8 g. Feeding, anaesthesia, infusion (saline, $[\text{3H}]\text{inulin}$, ^{32}P), surgical preparation and

clearances were as previously described [25a]. Systemic blood pressure was monitored throughout the experiments. Four groups of rats were studied. In group I, four rats were infused with (OH)Trp (50 $\mu\text{g}/\text{min}$ per 100 g body wt.) after a control period. Group II consisted of three rats infused with (OH)Trp as in group I; in addition, benzerazide (30 $\mu\text{g}/\text{min}$ per 100 g body wt., after a priming dose of 1.2 mg/100 g body wt. [8]), was infused throughout the experiment. Groups III and IV consisted of four and three rats respectively, infused with dihydro-ergotamine (DHE) (5 $\mu\text{g}/\text{min}$ per 100 g body wt.) after a control period. Benzerazide was also infused into group IV rats, as for group II.

Presentation of data

5-HT accumulation in the medium is expressed in nmol/ml. Intracellular cAMP content and uptake of P_i , MGP and alanine are expressed as pmol or nmol/mg of protein [31]. Na-dependent uptakes were calculated by subtracting uptake values measured in the presence of *N*-methyl-D-glucamine from those measured in the presence of Na. Results of *in vivo* experiments are expressed in terms of fractional P_i excretion in urine. Results are presented as means $\pm$ S.E.M. of three to five different experiments (*n*) in which duplicate results were obtained. One-way or two-way analyses of variance were performed and, when allowed by the *F* value, results were compared by the modified Dunnett *t*-test.

RESULTS

5-HT synthesis by OK cells

OK cells synthesized 5-HT from (OH)Trp (Figure 1). 5-HT accumulation in the extracellular medium increased linearly with the time of incubation up to 60 min (Figure 1A) and with increasing (OH)Trp concentration (Figure 1B) with a threshold of 1 μM . Benzerazide, an inhibitor of L-AAAD, at a concentration of 100 μM abolished 5-HT production.

Modulation of cAMP generation by 5-HT and (OH)Trp in OK cells

Because the presence of 5-HT_{1B} receptors, which are negatively linked to adenylate cyclase, has been reported in OK cells [21–24], we first confirmed that, in our hands, 5-HT affected cAMP generation in these cells. 5-HT up to 10 μM did not affect basal cAMP content (Figure 2A). However, 5-HT (1–1000 nM) decreased PTH-induced cAMP generation in a concentration-dependent manner. The inhibitory effect of 5-HT on PTH-stimulated cAMP generation did not occur when OK cells were pretreated for 18 h with 125 ng/ml pertussis toxin, indicating that a pertussis toxin-sensitive G-protein, most likely G_i , is involved in this inhibition.

The effect of (OH)Trp, the 5-HT precursor, on cAMP accumulation is shown in Figure 2(B). As with 5-HT, (OH)Trp did not affect the basal cAMP content, but decreased PTH-stimulated cAMP generation. The effect of (OH)Trp was significant from a concentration of 0.1 μM , i.e. one order of magnitude higher than the minimally effective 5-HT concentration. In order to discriminate between an intrinsic effect of (OH)Trp and the effect of 5-HT generated from (OH)Trp, we evaluated the inhibition induced by (OH)Trp in the absence or presence of 100 μM benzerazide. In the presence of this L-AAAD inhibitor, the (OH)Trp-induced decrease in the cAMP content was abolished (Figure 2B), suggesting that (OH)Trp acted through locally formed 5-HT.

Figure 2 Effect of 5-HT on intracellular cAMP content in OK cells

(A) OK cells were incubated for 20 min with the indicated concentrations of 5-HT in the absence ($\circ$, $\square$) or presence ($\bullet$, $\blacksquare$) of 10 nM PTH after pretreatment without ($\circ$, $\bullet$) or with ($\square$, $\blacksquare$) 125 ng/ml pertussis toxin for 18 h. Results are means $\pm$ S.E.M. from four different experiments in which duplicates were obtained. * Significantly different from the value with PTH alone ($P < 0.05$); § significantly different from the value without pertussis toxin ($P < 0.05$). (B) OK cells were incubated for 20 min with the indicated concentrations of (OH)Trp in the absence ($\circ$) or presence ($\bullet$, $\blacksquare$) of 10 nM PTH, without ($\circ$, $\bullet$) or with ($\square$, $\blacksquare$) benzerazide at a concentration of 100 μM . Results are means $\pm$ S.E.M. of four different experiments in which duplicates were obtained. * Significantly different from the value with PTH alone ($P < 0.05$); § significantly different from the value without benzerazide ($P < 0.05$).

Modulation of Na– P_i co-transport by 5-HT and (OH)Trp in OK cells

We next evaluated the effects of 5-HT and (OH)Trp on the activity of Na– P_i co-transport (Figure 3). As expected from previous studies [12,25a], PTH, at a concentration of 10 nM, decreased Na-dependent P_i uptake by 50%. This inhibition was reversed in a concentration-dependent manner by 5-HT (Figure 3A) and (OH)Trp (Figure 3B). Neither 5-HT nor (OH)Trp affected Na-dependent P_i uptake in the absence of PTH. Benzerazide, which had no influence on P_i uptake under control conditions or in the presence of PTH alone, abolished the effect of (OH)Trp (Figure 3B) but not that of 5-HT (Figure 3A).

The possibility that 5-HT affected the activity of Na– P_i co-transport by acting on the synthesis of new co-transporter was evaluated. The abundance of Na P_i -4 mRNA was measured by

Figure 3 Effects of 5-HT and (OH)Trp on Na-dependent P_i uptake by OK cells

OK cells were incubated for 3 h with 5-HT (A) or (OH)Trp (B) at the indicated concentrations in the absence (○) or presence (●, ■) of 10 nM PTH, without (○, ●) or with (■) 100 μ M benzerazide, prior to measurement of P_i uptake. Results are means $\pm$ S.E.M. of four different experiments in which duplicates were obtained. * Significantly different from the value with PTH alone ($P < 0.05$); § significantly different from the value without benzerazide ($P < 0.05$).

Figure 4 Effects of PTH and 5-HT on Na P_i -4 mRNA expression in OK cells

OK cells were incubated for 3 h with or without 10 nM PTH alone or in combination with 1 μ M 5-HT prior to extraction of RNA and Northern blot analysis, as described in the Materials and methods section. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Northern blotting in cells incubated with or without PTH and/or 5-HT. As shown in Figure 4, neither PTH alone nor the combination of PTH and 5-HT affected Na P_i -4 mRNA content.

Effects of 5-HT and (OH)Trp on Na–alanine and Na–MGP co-transport

In order to evaluate whether 5-HT affects Na– P_i co-transport selectively, we measured Na-dependent alanine and MGP uptake, which reflect the activity of co-transport systems previously

Table 1 Effects of 5-HT and (OH)Trp on Na-dependent uptake of alanine and MGP

OK cells were incubated for 3 h in the absence or in the presence of 10 nM PTH, alone or in combination with 5-HT or (OH)Trp, prior to measurement of alanine or MGP uptake. Results are means $\pm$ S.E.M. of three different experiments in which duplicates were obtained.

	Uptake (nmol/10 min per mg of protein)			
	Alanine		MGP	
	– PTH	+ PTH	– PTH	+ PTH
Basal	12.6 $\pm$ 0.4	13.4 $\pm$ 0.1	7.8 $\pm$ 0.2	9.0 $\pm$ 1.1
5-HT (1 μ M)	12.1 $\pm$ 0.9	11.1 $\pm$ 0.8	8.4 $\pm$ 0.5	7.7 $\pm$ 0.7
(OH)Trp (10 μ M)	12.8 $\pm$ 0.7	12.2 $\pm$ 0.8	8.1 $\pm$ 0.9	7.9 $\pm$ 0.6

demonstrated in OK cells. As shown in Table 1, 5-HT and (OH)Trp, alone or in combination with PTH, did not affect these transport systems.

Effects of 5-HT_{1B} receptor agonists on cAMP generation and Na– P_i co-transport in OK cells

It has been reported previously that DHE and L-propranolol behave as agonists towards 5-HT_{1B} receptors in OK cells [24]. Indeed, in our hands, both drugs decreased PTH-induced cAMP accumulation significantly (Table 2). In addition, DHE and L-propranolol attenuated significantly the inhibition by PTH of Na– P_i co-transport activity (Table 2). As shown in Figure 5, there is a good correlation between changes in intracellular cAMP content and changes in Na– P_i co-transport activity elicited by PTH, 5-HT, (OH)Trp, DHE and propranolol, which argues in favour of an instrumental role of cAMP in the modulation of P_i transport.

Modulation of urinary P_i excretion by (OH)Trp and DHE in rats

Systemic infusion of (OH)Trp resulted in a significant decrease of the fractional excretion of P_i , from 21.4 $\pm$ 1.3 % to 14.4 $\pm$ 1.0 % ($P < 0.05$) (Figure 6A). In agreement with recently reported data [8], infusion of benzerazide alone decreased P_i excretion, an effect most likely related to the inhibition of dopamine synthesis by renal tubular cells [8]. In the presence of benzerazide, (OH)Trp infusion had no effect on P_i excretion (Figure 6A). Neither (OH)Trp nor benzerazide affected significantly systemic blood pressure or renal haemodynamics, as judged from the stability of the glomerular filtration rate [1.1 $\pm$ 0.13, 1.0 $\pm$ 0.10 and 1.1 $\pm$ 0.11 ml/min per 100 g body wt. with vehicle, (OH)Trp and benzerazide respectively]. DHE infusion decreased the fractional excretion of P_i to the same extent as did (OH)Trp (Figure 6B). In the presence of benzerazide, the anti-phosphaturic effect of DHE persisted (12.2 $\pm$ 1.6 to 8.4 $\pm$ 1.2 %; $P < 0.01$).

Infusion of (OH)Trp may decrease renal dopamine synthesis by competition with L-dopa at the L-AAAD site, raising the possibility that the observed decrease in P_i excretion resulted from a decreased availability of phosphaturic dopamine. To test this hypothesis, we measured urinary dopamine excretion, which reflects renal dopamine synthesis, before and during (OH)Trp infusion. Dopamine excretion did not decrease during (OH)Trp infusion but, instead, increased from 11.9 $\pm$ 1.0 pmol/min to 25.1 $\pm$ 0.8 pmol/min ($P < 0.001$).

Table 2 Effects of DHE and L-propranolol on intracellular cAMP content and P_i uptake in OK cells

OK cells were incubated in the absence or presence of 10 nM PTH, alone or in combination with 1 μ M 5-HT, 10 nM DHE or 1 μ M L-propranolol, for 20 min or 3 h prior to determination of intracellular cAMP content and P_i uptake. Results are means $\pm$ S.E.M. of five different experiments in which duplicates were obtained. *Significantly different from the value with PTH alone ($P < 0.05$).

	Intracellular cAMP content (pmol/mg of protein)		Na-dependent P_i uptake (nmol/10 min per mg of protein)	
	– PTH	+ PTH	– PTH	+ PTH
Control	49.4 $\pm$ 6.2	556.3 $\pm$ 34.3	4.7 $\pm$ 0.3	1.9 $\pm$ 0.1
5-HT (1 μ M)	48.7 $\pm$ 5.2	201.0 $\pm$ 24.2*	4.6 $\pm$ 0.2	3.1 $\pm$ 0.2*
DHE (10 nM)	49.0 $\pm$ 6.4	286.6 $\pm$ 33.7*	4.5 $\pm$ 0.1	2.8 $\pm$ 0.2*
Propranolol (1 μ M)	49.0 $\pm$ 5.0	368.7 $\pm$ 85.8*	4.6 $\pm$ 0.2	2.7 $\pm$ 0.1*

Figure 5 Correlation between changes in cAMP content and in Na- P_i co-transport activity

Values of cAMP content and of Na-dependent P_i uptake are those shown in Figures 2 and 3 and in Table 1. Changes (Δ) were calculated as the value in the presence of the agonist(s) minus the value obtained under unstimulated conditions. The equation is $y = -0.008x + 0.13$; $r = 0.94$, $P < 0.001$.

DISCUSSION

In the present study, we have shown that: (i) 5-HT, synthesized by renal epithelial cells, exerts an autocrine/paracrine stimulation of Na- P_i co-transport through the occupation of 5-HT receptors negatively linked to adenylate cyclase in a pertussis toxin-sensitive manner; and (ii) *in vivo*, 5-HT and DHE, a 5-HT receptor agonist, are anti-phosphaturic in rats.

Proximal tubular cells have been shown to express high levels of L-AAAD, which converts L-dopa into dopamine and (OH)Trp into 5-HT [18–20]. Furthermore, tryptophan hydroxylase, which converts tryptophan into (OH)Trp, is also abundant in these cells which, therefore, have the ability to synthesize 5-HT from tryptophan, an essential amino acid [21]. That OK cells synthesize 5-HT could be expected from previous reports showing that these cells, which express a proximal phenotype, are able to synthesize dopamine [10,12]. It is noteworthy that conversion of (OH)Trp into 5-HT was quite efficient since, under our experimental conditions, approx. 20% of (OH)Trp (at 10 μ M) was converted into 5-HT after 30 min (Figure 1A). As regards the extracellular concentrations of the precursors used in the present study, they are consistent with the reported plasma tryptophan concentration in the rat, which is around 50 μ M [32].

As expected from previous studies, the inhibitory effect of 5-HT on cAMP generation was pertussis toxin-sensitive, a feature

consistent with the interaction between 5-HT and 5-HT_{1B} receptors. This receptor subtype has been extensively characterized in OK cells using pharmacological tools [22–24]. More recently, the cloning and expression of an OK cell cDNA encoding a 5-HT_{1B} receptor has been achieved [22]. Comparison of the amino acid sequence of this receptor with those of cloned 5-HT_{1B} receptors in other species has revealed 82% identity with mouse, rat and human receptors [21,33]. 5-HT_{1B} receptors have also been detected in the mouse kidney [34]. The highest density of receptors was detected in the outer medulla, which is consistent with the presence of receptors in the proximal straight tubule or pars recta, a major site involved in the regulation of P_i reabsorption by PTH [1,2].

That locally formed 5-HT modulates Na- P_i co-transport selectively in OK cells was confirmed by the following evidence: (i) the inhibitory effect of (OH)Trp on cAMP generation was abolished in the presence of benzerazide, an L-AAAD inhibitor (Figure 2); (ii) both 5-HT and (OH)Trp reversed the PTH-induced inhibition of P_i uptake; the effect of (OH)Trp, but not that of 5-HT, was reversed by benzerazide (Figure 3); (iii) 5-HT and (OH)Trp did not affect Na-dependent alanine and glucose transport, suggesting that the effect on Na- P_i co-transport was not a direct consequence of changes in the transmembrane Na gradient (Table 1). As regards the molecular and cellular mechanisms underlying the effect of 5-HT on the activity of Na- P_i co-transport, it is likely that phosphorylation/dephosphorylation reactions play a major role [2]. An effect of 5-HT on the synthesis of new Na- P_i co-transporter units, at least under our experimental conditions, could be ruled out by the absence of an effect of 5-HT on the abundance of NaP_i-4 mRNA, the Na- P_i isoform in OK cells [3] (Figure 4). A modulatory effect of 5-HT on Na- P_i co-transport has also been reported in HeLa cells, a non-renal epithelial cell line, transfected with 5-HT_{1A} receptors. In this model, 5-HT stimulated P_i uptake via a protein kinase C-dependent pathway [16]. It should be emphasized, however, that the consequences of protein kinase C activation on Na- P_i co-transport are dramatically different from one cell type to another: it stimulates Na- P_i co-transport in HeLa cells and in certain renal cell lines such as LLC-PK₁ and MDCK cells, while it decreases Na- P_i activity in OK cells and in proximal tubular cells in primary culture [2,29]. Moreover, mapping of 5-HT_{1A} receptors in the rat kidney revealed that they are not expressed in proximal tubular cells [16].

(OH)Trp induced changes in cAMP generation (Figure 2) and P_i transport (Figure 3) at concentrations lower than those necessary to observe a detectable increase in 5-HT accumulation in the extracellular medium (Figure 1). The reason for this

Figure 6 Effects of (OH)Trp and DHE on the fractional excretion of P_i (FE P_i) in rats

The FE P_i values for individual animals before and after the onset of administration of (OH)Trp or DHE are represented by two circles connected by a line. The means $\pm$ S.E.M. of results obtained from groups of animals that had been treated in the same way are represented by squares. * Significantly different from the control value ($P < 0.05$); § significantly different from the value without benserazide ($P < 0.05$).

discrepancy is not clear. It is very unlikely that (OH)Trp itself affects cAMP generation or P_i transport, since high concentrations of this substance failed to have any effect in the presence of benserazide (Figures 2 and 3). One possibility is that small amounts of 5-HT, large enough to stimulate 5-HT receptors but not to induce a detectable increase in the extracellular 5-HT concentration, are synthesized in the presence of 0.1–1 μ M (OH)Trp.

In vivo results obtained in rats were in complete agreement with those in OK cells, since we demonstrated the anti-phosphaturic effect of (OH)Trp infusion and the prevention of this effect by prior administration of benserazide. The observation that benserazide alone decreased P_i excretion is in line with a recent study showing that carbidopa, a dissimilar L-AAAD inhibitor, decreased P_i excretion in rats [8]. This effect of benserazide is most likely attributable to inhibition of dopamine synthesis, thus suppressing the phosphaturic effect of this amine [8]. Alternatively, the possibility that the anti-phosphaturic effect of (OH)Trp infusion was related to the decreased availability of L-dopa for dopamine synthesis by proximal cells needed to be evaluated, since it is likely that entry of (OH)Trp and L-dopa into

proximal cells occurs via the same transport systems and that competition between the two precursors may occur. This hypothesis, however, was not supported by the fact that urinary dopamine excretion, a reliable index of renal synthesis, was not decreased during (OH)Trp infusion. Instead, a slight but significant increase was observed. The cause of this increase is not clear; increased availability of L-dopa or increased activity of L-AAAD may be hypothesized.

DHE, an ergoline derivative previously shown to possess agonist activity towards the OK cell 5-HT_{1B} receptor [24], decreased P_i excretion in rats (Figure 6) and increased Na–P_i co-transport activity in OK cells (Table 2). These data further support the involvement of this receptor subtype in the modulation of renal P_i transport in the rat and may suggest that similar effects would occur in humans, in whom this drug has been used for many years. Furthermore, the observation that the anti-phosphaturic effects of DHE and benserazide were additive raises the possibility that such a therapeutic combination may prove efficient in reducing phosphaturia in patients with hypophosphataemia related to renal phosphate leakage.

In conclusion, we have demonstrated *in vitro* (in OK cells) and *in vivo* (in the rat kidney) that locally formed 5-HT is anti-phosphaturic through stimulating Na–P_i co-transport activity, a modulation exerted via 5-HT₁ receptors negatively coupled to adenylate cyclase in a pertussis toxin-dependent manner. These results emphasize the role of paracrine modulators of proximal origin, such as dopamine and 5-HT, in the fine tuning of renal phosphate excretion under physiological conditions and, most probably, in pathological situations characterized by abnormalities in the renal synthesis, effects and degradation of these bioactive amines.

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