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aminoaciduria
calcitonin
cyclic nucleotides
dietary hypocalcemia
parathyroid hormone
phosphaturia
vitamin D deficiency

Effect of Calciotropic Hormones and Cyclic Nucleotides on Aminoaciduria and Phosphaturia

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Summary

Parathyroid extract (PTE), dibutyrylcyclic AMP (dbcAMP), adenosine cyclic 3':5'-monophosphate (cAMP), calcitonin (CT), and calcium chloride were infused separately into anesthetized, sham-operated, or TPTX vitamin D-fed adult rats to examine the effect of these calciotropic agents on fractional excretion (FE) of α-aminoisobutyric acid (AIB), and phosphate anion (P_i). AIB is a nonmetabolizable amino acid.

Inulin clearance, FE_{AIB}, and FE_{P_i} were stable in the intact ($n = 10$) and TPTX rat ($n = 10$). TPTX decreased FE_{AIB}, and FE_{P_i} significantly ($P < 0.001$ for both). PTE and dbcAMP both increased FE_{AIB} in the intact rat ($P < 0.001$); failure to obtain this response in the TPTX animal was a key finding. PTE and dbcAMP increased FE_{P_i} ($P < 0.001$) in both the intact and TPTX animal. CT was the only agent (*versus* PTE, dbcAMP, adenosine cyclic 3':5'-monophosphate, and $CaCl_2$) to increase FE_{AIB} ($P < 0.001$) in the TPTX rat; furthermore, it was the only agent that did not increase FE_{P_i} in the TPTX rat although it had hypocalcemic and hypophosphatemic effects. Changes in inulin clearance or plasma concentration of AIB, following infusion of calciotropic agents, do not explain the unique responses in FE_{AIB} in the TPTX rat.

Our findings suggest that hyperaminoaciduria induced by parathyroid hormone and cyclic nucleotide in the intact animal may be mediated by CT. Hyperphosphaturia is not a necessary response to small-dose (25 milliunits/kg·hr) infusions of CT.

Speculation

Intracellular calcium in renal epithelium may be the final determinant of hyperaminoaciduria in hyperparathyroidism.

Net renal tubular reabsorption is impaired in man in the presence of vitamin D or primary calcium deficiency and in primary hyperparathyroidism (37). The tubular dysfunction includes excessive phosphaturia and aminoaciduria (17, 28, 33), bicarbonate loss (38, 39), and increased excretion of other solutes normally reabsorbed by the proximal tubule (12).

The origin of the tubulopathy is unknown. Alterations in renal hemodynamics (37, 38) or permeability characteristics of the proximal tubule (30, 31) related to an excess of PTH (1, 26) or cAMP (51, 52) may be responsible. However, excess PTH does not uniformly evince impaired reabsorption of solute. For example, there is no hyperaminoaciduria in two-thirds of patients with primary hyperparathyroidism (14). Muldowney *et al.* (39) noted no increase in amino acid excretion after giving PTE to normal subjects, and of four subjects with endogenous hyperparathyroidism or infused with PTH, only two had depressed amino acid reabsorption (48).

The above-mentioned observations suggest that increased PTH secretion *per se* is not enough to cause hyperaminoaciduria and that additional factors must be involved. We have examined the problem of PTH-related hyperaminoaciduria further by examining the effects of infused calciotropic hormones and cyclic nucleotides on tubular reabsorption of phosphate and of AIB a nonmetabolizable amino acid. Normocalcemic vitamin D-replete rats, either intact or acutely TPTX, were used in these studies.

MATERIALS AND METHODS

Female, Long-Evans hooded rats (190 to 220 g) were obtained from Canadian Breeding Farms, St. Eustache, Quebec, Canada and anesthetized with Inactin (100 mg/kg) (Henley & Co., N. Y.). Following dissection of the neck, the thyroid, and parathyroid glands were either left intact (sham operated) or removed (TPTX) by electric cautery.

Renal clearance studies began 4 to 6 hr following surgery when endogenous PTH and CT had been cleared from the circulation through at least five half-lives. An external jugular vein (for infusions) and a femoral artery (for blood sampling) were catheterized with polyethylene tubing, and the bladder was tied off to reduce dead space as described previously (36). The animals were infused with a solution containing 60 mM NaCl, 2.3 mM KCl, 1.3 g/dl inulin, and 400 μCi/liter inulin-methoxy ([^{3}H]methoxy, 50 to 150 μCi/g) at 16 ml/kg·hr, to achieve a steady-state of inulin clearance and urine flow following which, seven or eight consecutive clearance periods of 30 to 35 min each were obtained. Near the midpoint of the clearance periods, blood samples (80 to 130 μl each) were collected into heparinized capillary tubes. Inulin and AIB in plasma and hematocrit were measured in all periods; plasma phosphate was measured in periods 1, 3, 5, and 7. Urine inulin, phosphate, AIB, and urine volume were measured, and the animal was weighed in each clearance period.

INFUSION OF CALCIUM, CALCIOTROPIC HORMONES, AND CYCLIC NUCLEOTIDES

Infusions were performed with a Sage Model-355 Infusion Pump (Orion Res. Inc., Cambridge, MA). The syringe containing the test substance was inserted into the polyethylene tubing delivering the sustaining solution of electrolytes and inulin. Experimental infusions included: (1) calcium chloride, 0.66 mmoles/g·hr; (2) PTE, (100 USP units/ml; Lilly, Para-thor-mone) 40 units/g·hr; (3) purified porcine calcitonin (90 MRC units/mg, generously provided by Dr. Claude Arnaud), 25 or 500 munits/g· hr; (4) dbcAMP (Sigma Chemical Co., St. Louis, MO), 15 mg/g·hr; (5) cAMP (Sigma Chemical Co.), 5 mg/kg·hr. Nucleotides were dissolved in the infusion mixture no earlier than 30 min before their use. PTE was infused undiluted to maintain its stability. CT was dissolved in a solution containing 0.9% NaCl solution and acetic acid (0.01 mM) in the ratio 1:1 and human albumin (4 mg/ml). Less than 200 μl of this particular solution was infused per animal; vehicle alone was infused in the control periods.

RENAL CLEARANCE PROTOCOL

Three control clearance periods were obtained on each rat followed by four experimental clearance periods. This allowed each rat to serve as its own control, an approach necessitated by the important (3-fold) interanimal variation in FE_{AIB} (36).

ANALYTICAL METHODS

Established methods (see Ref. 36 for details) were used for liquid-scintillation counting, verification of the radiochemical purity of [1-^{14}C]AIB (New England Nuclear, Boston, MA) and inulin [^{3}H]methoxy (New England Nuclear) and the measurement of phosphate in plasma and urine.

STATISTICAL METHODS

Analysis of variance (49) was performed on the data for renal clearance, FE, and plasma concentrations of AIB and P_i. The rat, in our preparation, has excellent intraindividual stability of FE_{AIB}, FE_{P_i}, GFR, and plasma AIB under control conditions (36). Data in this report are presented as the mean ± S.E. of pooled interindividual differences for the means of the control (first three) clearance periods and for the difference in each experimental clearance period from the control mean to accommodate interindividual variation and permit pooled data to be analyzed from all rats studied under a specific protocol.

RESULTS

CONTROL DATA

FE_{AIB}, FE_{P_i}, and GFR were stable under control conditions (Fig. 1). FE_{AIB} and FE_{P_i} decreased following TPTX ($P < 0.001$), the change from control being greater for phosphate than for AIB. GFR was not altered by TPTX.

RESPONSE TO INFUSION OF PTE OR DBCAMP

Intact Animal. PTE given to the intact rat increased FE_{AIB} significantly ΔFE_{AIB}, +0.061 ± 0.016, mean ± S.E. after 60 min; $P < 0.001$) (Fig. 2). The renal response to PTE is mediated, at least partly, by the generation of cAMP (11). Accordingly, the effect of its analog dbcAMP was examined. FE_{AIB} was increased significantly by dbcAMP in the intact rat (ΔFE_{AIB}, +0.043 ± 0.006 after 60 min; $P < 0.001$) (Fig. 2).

FE_{P_i} increased significantly ($P < 0.001$) when either PTE or dbcAMP was given to the intact animal (Fig. 2).

PTE and dbcAMP had opposing effects on GFR on the intact sham-operated animal (Fig. 2), despite their similar effects on FE_{AIB} and FE_{P_i}. PTE produced a significant fall in GFR (ΔGFR −0.33 ± 0.06 after 60 min; $P < 0.001$) whereas dbcAMP increased it slightly ($P < 0.05$, first 30 min period only).

TPTX Animal. Neither PTE nor dbcAMP altered FE_{AIB} in the TPTX rat (Fig. 3). This is a key finding in the present study. Both agents increased FE_{P_i} significantly in the TPTX rat ($P < 0.001$) (Fig. 3).

The time course of the phosphaturic response to PTE was slower in the TPTX animals when compared to that of the intact rat (difference between ΔFE_{P_i} in the first treatment period of the two groups; $P < 0.001$), and the phosphaturia provoked by dbcAMP was greater in TPTX rats ($P < 0.001$) than in the intact animal given the same dose of nucleotide (namely, Fig. 3 *versus* Fig. 2). The greater filtered load of P_i in TPTX animals may explain the greater FE_{P_i} in the latter following dbcAMP.

GFR was decreased by PTE (ΔGFR, −0.13 ± 0.05 after 30 min; $P < 0.001$) and increased by dbcAMP (ΔGFR, +0.13 ± within 30 min, $P < 0.005$) (Fig. 3); these responses in GFR to the two agents are similar to those seen in the intact rat.

INFUSION OF cAMP AND CALCIUM: TPTX RAT

cAMP reduced FE_{AIB} in the TPTX rat (Fig. 3) (ΔFE_{AIB}, −0.032 ± 0.005 after 60 min; $P < 0.001$), increased FE_{P_i} significantly (ΔFE_{P_i}, +0.13 ± 0.001 after 60 min; $P < 0.001$), and reduced GFR (ΔGFR, −0.16 ± 0.05; $P < 0.005$).

The response to calcium (infused as $CaCl_2$) was similar to that following cAMP in the TPTX rat (Fig. 3). FE_{AIB} fell (ΔFE_{AIB}, −0.035 ± 0.007 after 30 min; $P < 0.01$), FE_{P_i} increased after 60 min (ΔFE_{P_i}, +0.017 ± 0.01; $P < 0.001$), and GFR fell (ΔGFR, −0.125 ± 0.04 after 30 min; $P < 0.01$). The change in phosphaturia

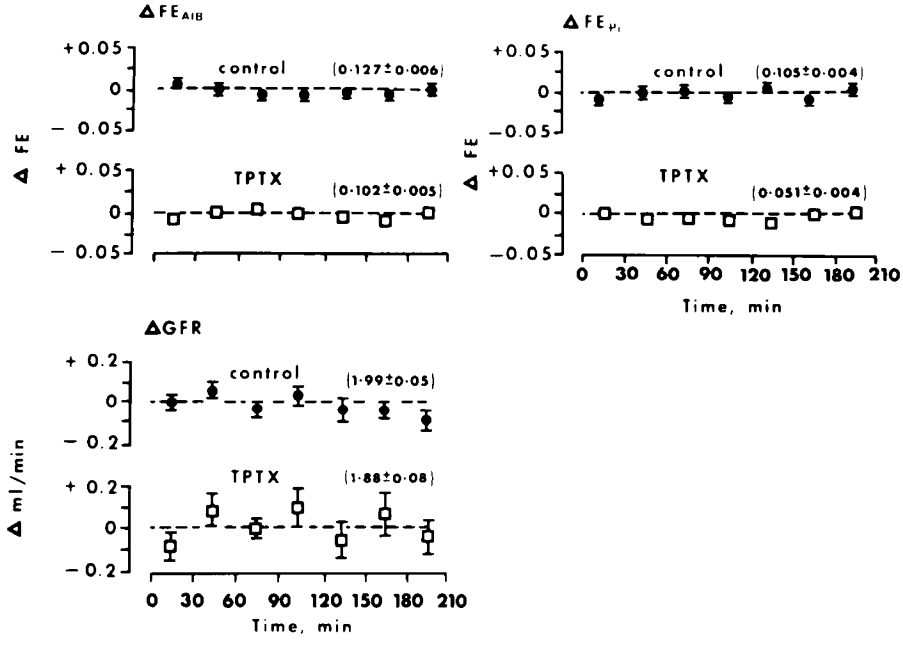

Fig. 1. The change in fractional excretion of the inert amino acid AIB and phosphate anion (ΔFE_{AIB} and ΔFE_{P_i}), and change in inulin clearance (ΔGFR) as a function of time in intact (n = 10) and TPTX (n = 10) rats. Data are presented as the average + S.E. of the difference between the mean of the first three clearance periods and each individual period for the individual rats. Interindividual differences between animals are thus accommodated. *Numbers above each set* of data are mean ± S.E. of absolute values for the group in periods one through three.

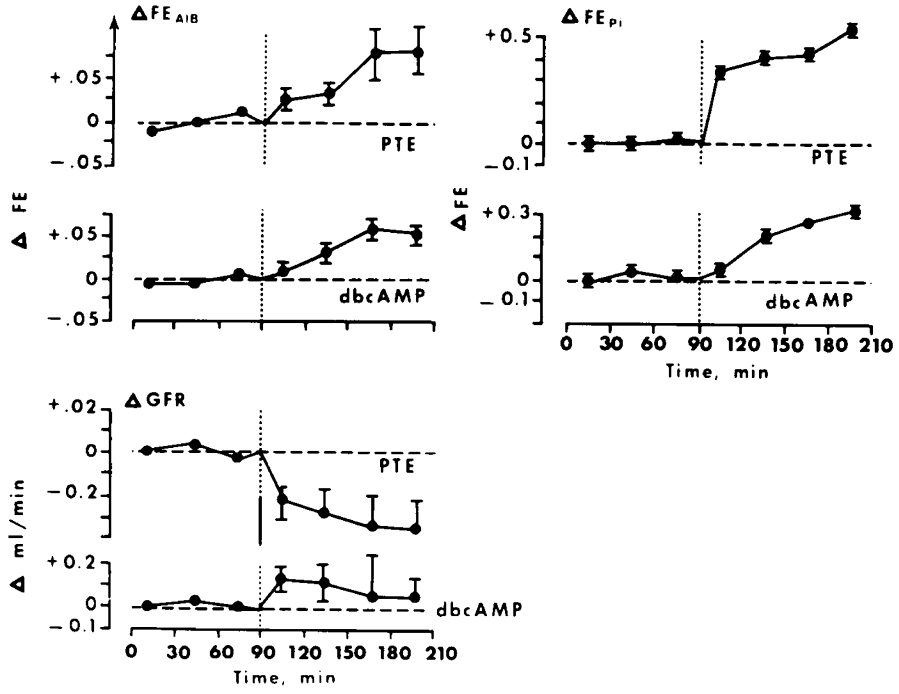

Fig. 2. ΔFE_{AIB}, ΔFE_{P_i}, and ΔGFR as a functon of time in the intact rat, infused with PTE ($n = 8$) or dbcAMP ($n = 6$). Data are presented as the difference (mean ± S.E.) between the mean of the first three (control) periods (- - - -) and each individual period (for both control or treatment clearance periods). Treatments (PTE or dbcAMP) began at 90 min and continued throughout periods 4 to 7 (90 to 120 min); the *vertical broken line* indicates onset of treatment.

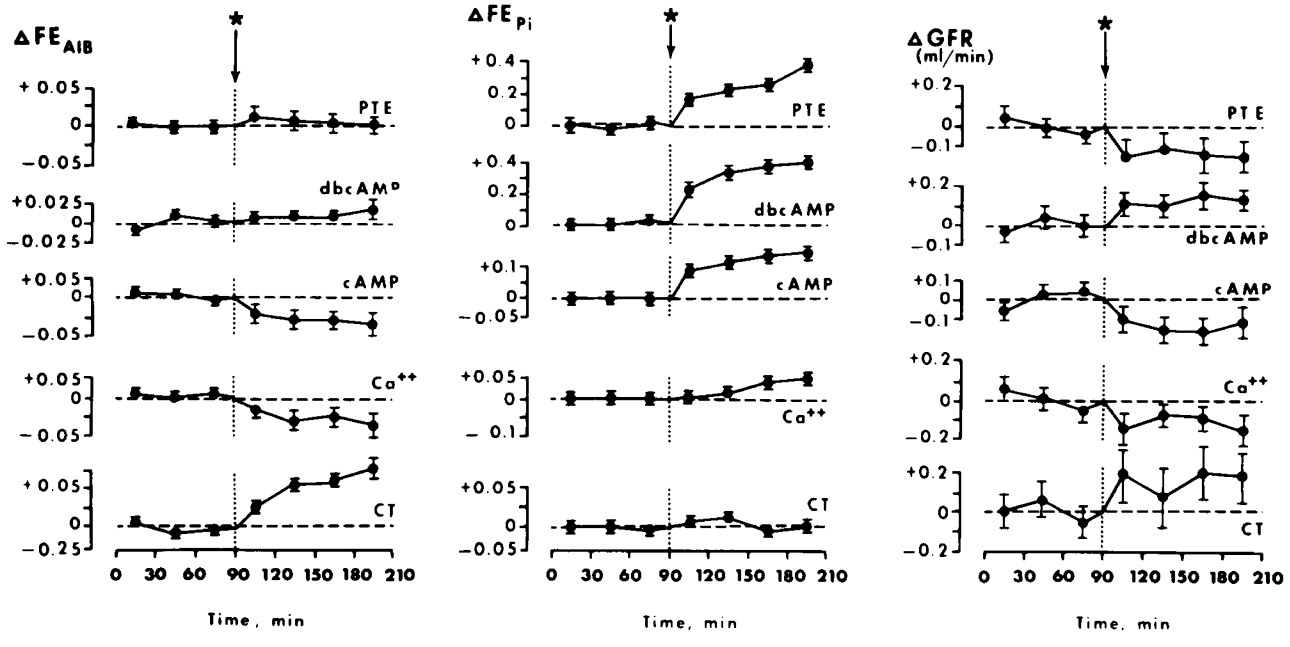

Fig. 3. ΔFE_{AIB}, ΔFE_{P_i}, and ΔGFR as a function of time in the TPTX rat group. Calciotropic agents and $CaCl_2$ were administered, and their effect was measured as explained in Figure 2. Number of animals in each group shown in Table 1.

could be attributed to known effects of concurrent hypercalcemia on phosphate metabolism (13).

RESPONSE TO CALCITONIN INFUSION: TPTX RAT

CT increased AIB excretion significantly (ΔFE_{AIB}, +0.064 ± 0.005 after 30 min; $P < 0.001$), but it had no effect on FE_{P_i} in the TPTX rat (Fig. 3). CT produced fluctuations in GFR, but this response was limited to two rats given 500 munits/kg·hr. In 5 rats given 25 munits/kg·hr, the control GFR was 2.04 ± 0.06 ml/min (mean ± S.E.; $n = 15$), and it did not change with CT infusion (2.06 ± 0.08 μl/min, mean ± S.E.; $n = 20$) during CT infusion; these five animals showed the aforementioned responses in FE_{AIB} and FE_{P_i}.

EFFECT OF CYCLIC NUCLEOTIDES AND HORMONES ON:

Plasma Calcium. The plasma level of calcium was stable in the intact and TPTX rat (Table 1). TPTX reduced plasma total calcium ($P < 0.001$), as expected. Infusion of PTE and dbcAMP into the intact rat had no effect on total plasma calcium; in the TPTX rat, PTE, cAMP, and $CaCl_2$ raised it significantly (see Table 1 for P values), dbcAMP had no effect, and CT lowered it ($P < 0.001$).

Plasma Phosphate. The plasma level of phosphate was stable in the intact and TPTX rat (Table 2). TPTX increased plasma phosphate ($P < 0.001$). The anticipated fall in plasma phosphate was observed following infusion of PTE and dbcAMP in both the intact and TPTX rat ($P < 0.001$). cAMP and CT reduced plasma phosphate ($P < 0.001$) in the TPTX rat, while $CaCl_2$ had no effect.

Plasma [AIB] and Filtered Load of AIB. Plasma [AIB] was stable in the intact and TPTX rat under control conditions (Fig. 4). Infusions of dbcAMP and CT into the intact and TPTX rat were each followed by significant changes in plasma [AIB]. The fall in plasma [AIB] following dbcAMP infusion in the intact rat ($P < 0.001$) (Fig. 4) was accompanied by a reciprocal increase in

Table 1. *Effect of hormones and nucleotides on plasma calcium (mg/dl)*

Period	Control		dbcAMP		PTE		CT TPTX	cAMP TPTX	$CaCl_2$ TPTX
	Intact	TPTX	Intact	TPTX	Intact	TPTX			
Preinfusion									
1	9.6 ± 0.2[1]	7.7 ± 0.2	10.0 ± 0.2	6.9 ± 0.4	9.0 ± 0.3	7.1 ± 0.3	7.3 ± 0.3	7.9 ± 0.2	8.2 ± 0.4
3	9.9 ± 0.3	7.4 ± 0.3	10.0 ± 0.3	7.7 ± 0.4	9.2 ± 0.4	6.9 ± 0.4	7.2 ± 0.4	7.7 ± 0.3	8.2 ± 0.4
Postinfusion[2]									
5	9.8 ± 0.3	7.4 ± 0.3	9.8 ± 0.3	7.7 ± 0.3	9.2 ± 0.5	7.5 ± 0.4	6.2 ± 0.3	8.4 ± 0.3	9.9 ± 0.4
7	10.0 ± 0.4	7.3 ± 0.3	9.8 ± 0.4	7.4 ± 0.4	8.9 ± 0.3	9.4 ± 0.6	5.8 ± 0.3	9.2 ± 0.5	11.9 ± 0.2
n	10	10	6	8	8	10	7	7	7
Significance of change[3]	n.s.[4]	n.s.	n.s.	n.s.	n.s.	$P < 0.005$	$P < 0.001$	$P < 0.01$	$P < 0.001$

[1] Mean ± S.E.
[2] No infusion was given to rats reported in first two columns.
[3] Tests of significance were determined by analysis of variance (48) for preinfusion periods *versus* postinfusion periods.
[4] n.s., not significant.

Table 2. *Effect of hormones and nucleotides on plasma phosphate (mg/dl)*

Period	Control		dbcAMP		PTE		CT TPTX	cAMP TPTX	$CaCl_2$ TPTX
	Intact	TPTX	Intact	TPTX	Intact	TPTX			
Preinfusion									
1	5.7 ± 0.2[1]	8.5 ± 0.3	6.2 ± 0.4	9.1 ± 0.7	5.7 ± 0.3	8.5 ± 0.2	7.9 ± 0.3	9.1 ± 0.4	8.3 ± 0.21
2	5.8 ± 0.2	8.9 ± 0.2		9.1 ± 0.7	5.8 ± 0.3		8.1 ± 0.3	8.8 ± 0.4	
3	5.8 ± 0.2	8.6 ± 0.2	6.0 ± 0.3	8.7 ± 0.7	5.8 ± 0.3	8.5 ± 0.3	7.9 ± 0.3	8.5 ± 0.3	8.2 ± 0.4
Postinfusion[2]									
4	5.9 ± 0.2	8.5 ± 0.1		7.9 ± 0.3	4.9 ± 0.3		7.1 ± 0.2	7.9 ± 0.4	
5	5.8 ± 0.2	8.5 ± 0.2	5.1 ± 0.3	6.3 ± 0.4	4.3 ± 0.3	6.9 ± 0.3	6.7 ± 0.2	7.1 ± 0.5	8.6 ± 0.3
6	5.9 ± 0.2	8.5 ± 0.1		5.9 ± 0.4	4.2 ± 0.2		6.4 ± 0.2	6.7 ± 0.5	
7	5.8 ± 0.2	8.2 ± 0.2	4.3 ± 0.2	5.9 ± 0.4	3.9 ± 0.2	6.0 ± 0.3	6.3 ± 0.2	6.1 ± 0.3	8.6 ± 0.2
Statistical significance[3]	n.s.	n.s.	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	n.s.

[1] Mean ± S.E.
[2] No infusion was given to rats reported in first two columns.
[3] Tests of significance were determined by analysis of variance (48) for preinfusion periods *versus* postinfusion periods.
[4] n.s., not significant.

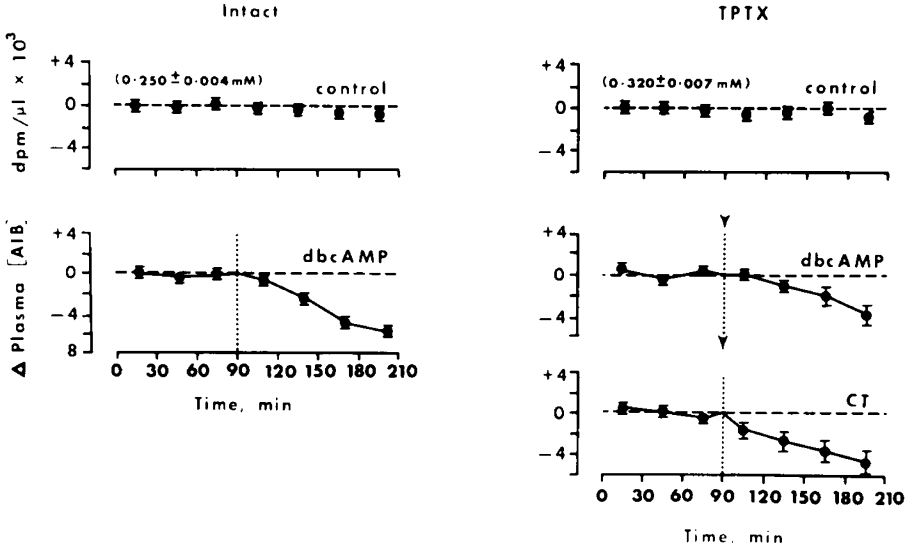

Fig. 4. Change in plasma [AIB] (Δplasma [AIB]) as a function of time in the intact and TPTX rat under control conditions and after infusion of dbcAMP and CT, and their effect measured as explained in Figure 2. Number of animals in each group shown in Table 1.

FE_{AIB} (Fig. 2); the latter was not accounted for by significant net change in filtered load because GFR increased during the above-mentioned changes in plasma [AIB] and FE_{AIB}. Despite a similar fall in plasma [AIB] ($P < 0.001$) in the TPTX rat following dbcAMP infusion (Fig. 4), and a similar increase in GFR (Fig. 3), FE_{AIB} did not increase. On the other hand, CT both decreased plasma [AIB] (Fig. 4) and increased FE_{AIB} (Fig. 3) in the TPTX rat.

DISCUSSION

PTE increased renal excretion of AIB in the intact, vitamin D-replete animal in our study. Micropuncture in the intact rat was used previously by Gekle (19) to confirm that PTH will decrease net reabsorption of natural amino acids *in situ*. These findings seem to support the hypothesis that the hyperaminoaciduria associated with deficiency of vitamin D (17) or calcium (28, 33) in both man and the rat (23) is caused by the hyperparathyroidism that occurs with these hypocalcemic conditions. However, other forms of hyperparathyroidism in man are not uniformly associated with impaired renal reabsorption of amino acids (14, 39, 48). An explanation for the irregular effect of PTH on tubular reabsorption of amino acids and for its mechanism of action in causing hyperaminoaciduria is offered by the study reported here.

The failure to increase renal excretion of AIB following infusions of PTE and dbcAMP in the TPTX rat is the key observation in our investigation. Because prolongation of the PTE infusion up to 12 hr in the TPTX rat, and EGTA infusion sufficient to cause hyperparathyroidism in the intact animal (35) both failed to elicit hyperaminoaciduria, we must conclude that hyperparathyroidism by itself is an insufficient condition for impaired tubular reabsorption of amino acids.

A permissive effect of the thyroid gland on the genesis of hyperaminoaciduria following infusions of PTE and dbcAMP is indicated. Increased secretion of either thyroxin or calcitonin could be involved. PTH itself is not expected to alter thyroid hormone secretion. Although secretion of thyroid hormone is an adenyl cyclase-dependent mechanism, which can be stimulated by dibutyryl cAMP (16), the rapid change in AIB excretion following dbcAMP is inconsistent with the characteristically slow response of cellular events to the hormone (22). Furthermore, hyperthyroidism is not known to cause hyperaminoaciduria (47).

Increased secretion of calcitonin follows exposure of the thyroid gland to the dbcAMP, both *in vitro* (4) and *in vivo* (8). Although PTH does not stimulate calcitonin secretion directly, its hypercalcemic effect is a potent stimulus for CT release experimentally (43). Persistence of normocalcemia in the PTE-infused intact animal is evidence that calcitonin released by the thyroid gland, could have been significant in our experiments; plasma calcium was increased by PTE only in the TPTX rat in our experiments.

Calcitonin promptly increased AIB excretion in the TPTX rat. We observed a similar effect in the single intact animal that we studied (35). The small but significant decrease in baseline FE_{AIB} in the TPTX rat is also consistent with an effect of CT *in vivo* at physiologic concentrations. Small infusions of CT (25 munits/kg·hr) did not alter GFR or urine flow rate while FE_{AIB} was increasing. Other workers (42) have shown that CT does not alter renal plasma flow. Thus, there is no evidence that the increase in AIB excretion induced by CT is secondary to altered renal hemodynamics.

The lack of a phosphaturic effect following CT infusion in our TPTX (and intact) rats concurs with some studies (50), but conflicts with others (41). The reasons for these varying results are unclear (2), but some of the differences may be dose related, an impression supported by Robinson *et al.* (45). For example, although the effect on amino acid excretion in our studies was obtained with 25 munits/kg·hr of porcine Ct, the dose used to achieve phosphaturia with the more potent salmon in other experimental (41) was ~3200-fold larger. We suggest that induction of phosphaturia may be a pharmacologic rather than a physiologic action of CT.

It is not known whether the renal effects of CT are mediated by cAMP. CT-responsive adenyl cyclase activity has been identified largely in the medullary and cortical portions of the thick ascending limb and in the distal convoluted tubule (9). Whether these regions of the nephron could be the sites of decreased AIB uptake from the lumen or of an increased cell-to-lumen flux of AIB (36) remains to be determined.

Borle (6) suggests that a major effect of CT is to lower the cytoplasmic calcium ion concentration. A depression of cytoplasmic calcium has been found in renal cells of vitamin D-deficient chicks (7). A role for cytoplasmic calcium in the control of membrane permeability has been recognized in other tissues (7, 34, 44). Increased permeability to ions and other solutes follows elevation of cytoplasmic calcium (3, 21, 29, 46). Consequently, a depression of renal cytoplasmic calcium might decrease permeability of the brush border membrane, thereby impairing net reabsorption of amino acids. Therefore, depletion of cell calcium stores, with a fall in cytoplasmic calcium ion and membrane permeability, might be the common denominator underlying the tubular dysfunction in calcium deficiency, vitamin D deficiency, and isolated deficiency of 1,25-dihydroxycholecalciferol (15). We can speculate further that decreased cytoplasmic calcium ion might occur in some target organs in primary hyperparathyroidism because of increased calcitonin release (24) and relative deficiency of vitamin D hormone (32, 54).

There is some evidence that alterations in renal hemodynamics may play a role in the tubulopathy of primary and secondary hyperparathyroidism (37). This is a particular problem when PTE is used instead of PTH, as was the case in our studies. Perturbation of renal hemodynamics by PTE was indicated by the fall in GFR and urine flow rate in our intact and TPTX rats. However, it is unlikely that these changes are responsible for increased FE_{AIB} in the intact PTE-infused rat because FE_{AIB} in TPTX rats was unaffected by PTE, despite changes in GFR and flow rate analogous to those found in intact animals. With dbcAMP infusion, an increase in GFR and urine flow rate occurred in the intact and TPTX animal, yet AIB excretion still increased in the intact group. Thus, while PTE and dibutyryl cAMP both increase FE_{AIB} in intact rats, they have divergent effects on GFR.

Further evidence against a simple relationship between GFR and FE_{AIB}, in response to calciotropic agents, is found in our studies with cAMP and $CaCl_2$. Decreased renal blood flow and GFR in response to cAMP has been described previously (20, 27). Although the fall in GFR induced by calcium may be PTH dependent (25), we still observed this response in TPTX rats. Because GFR decreased in situations in which FE_{AIB} either fell (calcium and cAMP infusions in TPTX rats), remained unchanged (PTE infusion in TPTX rats), or rose (PTE infusion in the intact rat), or GFR increased in situations in which FE_{AIB} either rose (dibutyryl cAMP infusion in the intact rat) or remained unchanged (dbcAMP infusion in TPTX rats), we must conclude that hemodynamic changes are an insufficient explanation for the changes in FE_{AIB} in response to calciotropic agents.

It is probable that the alterations in GFR induced by PTE are not the effect of parathyroid hormone itself but rather of contaminants in the extract (2). Dibutyryl cAMP has been reported to be without effect on the renal vasculature of the dog (20), but other studies suggest that it has vasodilatory actions (5, 27) which could be consistent with the effects we observed.

Finally, there is a particular effect of dbcAMP to be considered. Plasma [AIB] fell in the intact and TPTX rat treated with the nucleotide. This change cannot be related to increased urinary loss of AIB alone, although the rapid decrease in plasma [AIB] in the intact rat may reflect the increased renal excretion of amino acid. Enhanced AIB uptake by liver and other organs (10, 39, 53) is probably an important cause for the fall in plasma [AIB] following treatment with dbcAMP. However, this analog depletes kidney of AIB *in situ* (18), a decrease which could reflect increased flux of AIB from cells to lumen (36).

CONCLUSION

Hyperparathyroidism has been proposed as an essential cause of hyperaminoaciduria and hyperphosphaturia in vitamin D de-

ficiency and dietary hypocalcemia. However, primary hyperparathyroidism is not uniformly accompanied by hyperaminoaciduria. This discrepancy has been investigated further in a rat model using the inert amino acid AIB as a probe of amino acid reabsorption *in vivo*. We observed that PTE and dbcAMP increased FE_{AIB} and FE_{Pi} in the intact, sham-operated rat whereas neither agent increased FE_{AIB} in the TPTX animal. In the latter, small doses of CT (25 munits/kg·hr) increased FE_{AIB} significantly without change in FE_{Pi}. These findings indicate different mechanisms for hyperaminoaciduria and hyperphosphaturia in response to calciotropic agents. We propose that net reabsorption of amino acids in hyperparathyroid states is modulated by CT, perhaps through its effect on cytosol calcium and membrane permeability of renal tubular epithelium.

REFERENCES AND NOTES

1. Arnaud, C., Glorieux, F., and Scriver, C. R.: Serum parathyroid hormone levels in acquired vitamin D deficiency or infancy. Pediatrics, *49:* 837 (1972).
2. Aurbach, G. D., and Heath D. A.: Parathyroid hormone and calcitonin regulation of renal function. Kidney Int., *6:* 331 (1974).
3. Baker, P. F.: Transport and metabolism of calcium ions in nerve. In: J. A. V. Butler, D. Noble: Progress in Biophysics and Molecular Biology. Vol. 24, p. 179 (Pergamon Press, New York, 1972).
4. Bell, N. H., and Queener, S.: Stimulation of calcitonin synthesis and release in vitro by calcium and dibutyryl cyclic AMP. Nature (Lond.), *248:* 343 (1974).
5. Berti, F., Bernareggi, V., and Mandelli, V.: Contraction and relaxation of in vitro perfused rat caudal artery: a possible role for cyclic 3'5'-AMP. Arch. Int., Pharmacodyn. Ther., *192:* 247 (1971).
6. Borle, A. B.: Calcium metabolism at the cellular level. Fed. Proc. *32:* 1944 (1973).
7. Borle, A. B.: Kinetic studies of calcium movements in intestinal cells: effects of vitamin D deficiency and treatment. J. Membr. Biol., *16:* 207 (1974).
8. Care, A. D., Bates, R. F. L., and Gitelman, H. J.: A possible role for the adenyl cyclase system in calcitonin release. J. Endocrinol., *48:* 1 (1970).
9. Chabardes, D., Imbert-Teboul, M., Montegut, M., Clique, A., and Morel, F.: Distribution of calcitonin-sensitive adenyl cyclase activity along the rabbit kidney tubule. Proc. Natl. Acad. Sci. U. S. A., *73:* 3608 (1976).
10. Chambert, J. W., Georg, R. H., and Bass, A. D.: Effect of glucagon, cyclic 3',5'-adenosine monophosphate and its dibutyryl derivative on amino acid uptake by the isolated perfused rat liver. Endocrinology, *87:* 366 (1970).
11. Chase, L. R., and Aurbach, G. D.: Parathyroid function and renal excretion of 3',5'-adenylic acid. Proc. Natl. Acad. Sci. U. S. A., *58:* 518 (1967).
12. Chesney, R. W., and Harrison, H. E.: Fanconi syndrome following bowel surgery and hepatitis reversed by 25-hydroxycholecalciferol. J. Pediatr., *86:* 857 (1975).
13. Cuche, J. L., Ott, C. E., Marchand, G. R., Diaz-Buxo, J. A., and Knox, F. G.: Intrarenal calcium in phosphate handling. Am. J. Physiol., *230:* 790 (1976).
14. Cusworth, D. C., Dent, C. E., and Scriver, C. R.: Primary hyperparathyroidism and hyperaminoaciduria. Clin. Chim. Acta, *41:* 355 (1972).
15. Drezner, M. K., and Feinglos, M. N.: Osteomalacia due to 1α,25-dihydroxycholecalciferol deficiency. J. Clin. Invest., *60:* 1046 (1977).
16. Dumont, J. E.: The action of thyrotropin on thyroid metabolism. Vitam. Horm., *29:* 287 (1971).
17. Fraser, D., Kooh, S. W., and Scriver, C. R.: Hyperparathyroidism as the cause of hyperaminoaciduria and phosphaturia in human vitamin D deficiency. Pediatr. Res., *1:* 425 (1967).
18. Fuller, R. W., and Baker, J. C.: Effect of glucagon and dibutyryl cyclic AMP on the tissue distribution of [^{14}C]α-aminoisobutyric acid in rats. FEBS Lett., *53:* 8 (1975).
19. Gekle, D.: The influence of parathyroid hormone on renal function. Pflugers Arch. Eur. J. Physiol., *323:* 96 (1971).
20. Gill, J. R., Casper, A. G. T., and Tate, J.: Renal effects of adenosine 3',5'-cyclic monophosphate and dibutyryl adenosine 3',5'-monophosphate. J. Clin. Invest., *50:* 1231 (1971).
21. Godfraind, J. M., Kawamura, H., Krnjevic, K., and Pumain, P.: Action of dinitrophenol and some other metabolic inhibitors on cortical neurons. J. Physiol. (Lond.), *215:* 199 (1971).
22. Gordon, P., Robbins, J., and Rall, J. E.: The thyroid and iodine metabolism. In: P. K. Bondy, L. E. Rosenberg: Duncan's Diseases of Metabolism. 7th ed. Chap. 17, (W. B. Saunders, Philadelphia, 1974).
23. Grose, J. H., and Scriver, C. R.: Parathyroid-dependent phosphaturia and aminoaciduria in the vitamin D-deficient rat. Am. J. Physiol., *214:* 370 (1968).
24. Heynen, G., and Franchimont, P.: Human calcitonin radioimmunoassay in normal and pathological conditions. Eur. J. Clin. Invest., *4:* 213 (1974).
25. Humes, H. D., Ichikawa, I., Troy, J. L., and Brenner, B. M.: Evidence for a parathyroid hormone-dependent influence of calcium on the glomerular ultrafiltration coefficient. J. Clin. Invest., *61:* 32 (1978).
26. Joffe, B. I., Hackens, W. H. L., Seftel, H. C., and Hartdegen, R. G.: Parathyroid hormone concentrations in nutritional rickets. Clin. Sci. (Oxf.), *42:* 113 (1972).
27. Kokubun, H.: Direct effects of cyclic AMP and dibutyryl cyclic AMP on renal blood flow. Tohoku J. Exp. Med., *116:* 295 (1975).
28. Kooh, S. W., Fraser, D., Reilly, B. J., Hamilton, J. R., Gall, D. G., and Bell, L.: Rickets due to calcium deficiency. N. Engl. J. Med., *297:* 1264 (1977).
29. Lew, V. L.: Effect of intracellular calcium on the potassium permeability of human red cells. J. Physiol. (Lond.), *206:* 35 (1970).
30. Lorentz, W. B.: The effect of cyclic AMP and dibutyryl cyclic AMP on the permeability characteristics of the renal tubule. J. Clin. Invest., *53:* 1250 (1974).
31. Lorentz, W. B.: Effect of parathyroid hormone on renal tubular permeability. Am. J. Physiol., *231:* 1401 (1976).
32. Lumb, G. A., and Stanbury, S. W.: Parathyroid function in human vitamin D deficiency and vitamin D deficiency in primary hyperparathyroidism. Am. J. Med., *56:* 833 (1974).
33. Maltz, H. E., Fish, M. B., and Holliday, M. A.: Calcium deficiency rickets and the renal response to calcium infusion. Pediatrics, *46:* 865 (1970).
34. Manery, J. F.: Effects of calcium ions on membranes. Fed. Proc., *25:* 1804 (1966).
35. R. R. McInnes, unpublished observations.
36. McInnes, R. R., and Scriver, C. R.: Net reabsorption of α-aminoisobutyric acid by rat kidney *in vivo*. Am. J. Physiol., *237:* F274 (1979).
37. Morris, R. C., McInnes, R. R., Epstein, C. J., Sebastian, A., and Scriver, C. R.: In: B. M. Brenner, F. C. Rector: The Kidney. Chap. 27, p. 1204 (W. B. Saunders, Philadelphia, 1976).
38. Morris, R. C., Sebastian, A., and McSherry, E.: Renal acidosis. Kidney Int., *1:* 322 (1972).
39. Muldowney, F. P., Freaney, R., and McGeeney, D.: Renal tubular acidosis and aminoaciduria in osteomalacia of dietary or intestinal origin. Q. J. Med., *37:* 517 (1968).
40. Phang, J. M., and Downing, S. J.: Amino acid transport in bone: stimulation by cyclic AMP. Am. J. Physiol., *224:* 191 (1973).
41. Popovtzer, M. M., Blum, M. S., and Flis, R. S.: Evidence for interference of 25-(OH) vitamin D_3 with phosphaturic action of calcitonin. Am. J. Physiol., *232:* E515 (1977).
42. Puschett, J. B., Beck, W. S., Jr., Jelonek, A., and Fernandez, P. C.: Study of the renal tubular interactions of thyrocalcitonin, cyclic adenosine 3',5'-monophosphate, 25-hydroxycholecalciferol, and calcium ion. J. Clin. Invest., *53:* 756 (1974).
43. Queener, S. F., and Bell, N. H.: Calcitonin: A general survey. Metabolism, *24:* 555 (1975).
44. Rasmussen, H., and Bordier, P.: The Physiological and Cellular Basis of Metabolic Bone Disease. Chap. 4, p. 105 (Williams & Wilkins Co., Baltimore, 1974).
45. Robinson, C. J., Martin, T. J., and MacIntyre, I.: Phosphaturic effect of thyrocalcitonin. Lancet, *2:* 83 (1966).
46. Romero, P. J., and Whittam, R.: The control by internal calcium of membrane permeability to sodium and potassium. J. Physiol. (Lond.), *214:* 481 (1971).
47. Scriver, C. R., and Rosenberg, L. E.: Amino Acid Metabolism and its Disorders. (W. B. Saunders, Philadelphia, 1973).
48. Short, E. M., Elsas, L. J., and Rosenberg, L. E.: Effect of parathyroid hormone on renal tubular reabsorption of amino acids. Metabolism, *23:* 715 (1974).
49. Sokal, R. R., and Rohlf, F. J.: Biometry. (W. H. Freeman and Co., San Francisco, 1969).
50. Sorensen, O. H., and Hindberg, I.: The acute and prolonged effect of porcine calcitonin on urine electrolyte and excretion in intact and parathyroidectomized rats. Acta Endocrinol., *70:* 295 (1972).
51. Sovik, O., Aksnes, L., and Apold, J.: Urinary cyclic AMP: high concentrations in vitamin D-deficient and -dependent rickets. J. Pediatr. *89:* 946 (1976).
52. Vainsel, M., Manderlier, T., and Otten, J.: Urinary excretion of adenosine 3',5'-monophosphate in vitamin D deficiency. Eur. J. Clin. Invest., *6:* 127 (1976).
53. Weiss, I. W., Morgan, K., and Phang, J. M.: Cyclic adenosine monophosphate-stimulated transport of amino acids in kidney cortex. J. Biol. Chem., *247:* 760 (1972).
54. Woodhouse, N. S. Y., Doyle, F. H., and Joplin, G. F.: Vitamin-D deficiency and primary hyperparathyroidism. Lancet, *2:* 283 (1971).
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