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Increase in membrane fluidity modulates sodium-coupled uptakes and cyclic AMP synthesis by renal proximal tubular cells in primary culture

Gérard Friedlander, Christian Le Grimellec and Claude Amiel

INSERM U 251 and Department of Physiology, Faculté de Médecine Xavier-Bichat, Université Paris 7, Paris (France)

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In order to evaluate the influence of membrane fluidization on three apical transport systems and on a basolateral enzyme, and to analyse the mechanisms involved, we studied, in cultured rabbit proximal tubular cells, the effect of increasing concentrations of the local anesthetic drug benzyl alcohol on Na^+-dependent uptakes of phosphate (P_i), methyl α-D-glucopyranoside (MGP), and L-alanine, as well as on basal and stimulated cyclic AMP content. At 10 mM, benzyl alcohol increased the V_{max} of P_i uptake by 31%, decreased that of MGP uptake by 24%, and did not affect alanine uptake. K_m values were not affected. Benzyl alcohol, up to 40 mM, increased in a concentration-dependent manner basal, PTH-stimulated, and cholera toxin-stimulated, but not forskolin-stimulated cyclic AMP accumulation. In the presence of 40 mM benzyl alcohol, the magnitude of PTH-induced inhibition of P_i uptake was enhanced from 11% to 24%. It is concluded that: (i) fluidization of apical membranes affected differently Na^+/P_i, Na^+/MGP, and Na^+/alanine cotransports, reflecting differences in the lipidic environments of these transport system; (ii) fluidization of basolateral membranes enhanced PTH-stimulated cyclic AMP generation through improved coupling between the receptor-G_S complex and the catalytic subunit of adenylate cyclase; (iii) these variations may result in physiological and pathophysiological modulation of the renal handling of solutes and of the phosphaturic effect of PTH.

Introduction

Modulation of membrane fluidity has been shown to affect the activity of numerous membrane-bound proteins, in particular transport systems and enzymes [1]. In renal proximal tubular cells, apically located transporters such as Na^+-P_i and Na^+-glucose were shown to be very sensitive to changes in the physical state of their lipidic environment [2,3]. We have recently shown that, in intact renal epithelial cells, increasing the fluidity of apical membranes, either with low concentrations of the local anesthetic drug benzyl alcohol [4] or by opening of tight junctions [5,6], resulted in an increase in P_i uptake and a decrease of hexose uptake [7,8]. The activity of renal adenylate cyclase, an enzyme located in the basolateral membrane in polarized cells such as epithelial ones, was also shown to be influenced by changes in membrane fluidity in preparations of basolateral membranes [9] as well as in intact renal cells [4]. Indeed, PTH-induced stimulation of adenylate cyclase in basolateral membranes from dog renal cortex was markedly enhanced in the presence of benzyl alcohol [9]. However, the possibility that membrane fluidization might modulate PTH-induced inhibition of P_i transport in the proximal tubule has not been evaluated.

The aim of the present study was to examine the influence of membrane physical state on apical Na^+-coupled uptakes of P_i, hexose, and alanine, as well as on basal and stimulated cAMP generation and on PTH-induced inhibition of P_i uptake by rabbit proximal tubular cells grown in primary cultures, and to analyse the mechanisms involved. We show that benzyl alcohol-induced membrane fluidization: (i) stimulated P_i uptake, depressed hexose uptake and did not affect alanine uptake; (ii) increased the generation of cAMP through improved coupling between the receptor-regulatory protein complex and the catalytic subunit of adenylate cyclase; and (iii) enhanced the inhibitory effect of PTH

Abbreviations: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; HBS, Hanks' balanced salt solution; IBMX, 3-isobutyl-1-methylxanthine; MGP, methyl α-D-glucopyranoside; bPTH 1–34, 1–34 fragment of bovine parathyroid hormone.

Correspondence: G. Friedlander, INSERM U 251, Faculté de Médecine Xavier-Bichat, 16, rue Henri Huchard, F-75018, Paris, France.

on P_i uptake through increased PTH-induced cAMP production.

Experimental procedures

Materials

Ethanolamine, insulin, transferrin, dexamethasone, triiodothyronine, methyl α-D-glucopyranoside (MGP), L-alanine, sodium, selenite, 3-isobutyl-1-methylxanthine (IBMX), 2′-*O*-dibutyryladenosine 3′:5′ cyclic monophosphate (dibutyryl-cAMP), type I collagenase, bovine serum albumin, cholera toxin, and the 1–34 fragment of bovine parathyroid hormone (bPTH 1–34) were purchased from Sigma Chemical (St Louis, MO, U.S.A.). Forskolin was from Calbiochem-Behring (La Jolla, CA, U.S.A.). Percoll was purchased from Pharmacia AB (Uppsala, Sweden). Tracers were from the following sources: $K_2H^{32}PO_4$ from New England Nuclear (Boston, MA, U.S.A.), carrier-free $Na^{125}I$, methyl α-D-[U-^{14}C]glucopyranoside [^{14}C]MGP, and L-[2,3-^{3}H]-alanine from Amersham (Amersham, U.K.). Culture media and reagents were from Flow Labs (Irvine, U.K.). Plasticware was from Falcon (Oxnard, CA, U.S.A.). All other reagents were of analytical grade.

Methods

Cell culture. Primary cultures of renal proximal tubular cells were prepared according to Bello-Reuss et al. [10] with minor modifications, as described previously [7]. Briefly, kidneys were removed aseptically from anesthetized New Zealand rabbits (0.9–1 kg), decapsulated and sliced in 1-mm thick sections which were kept at 4° C in Hank's balanced salt solution additioned with 10 mM Hepes and 5 mM D-glucose (pH = 7.4) (HBS-Hepes). Cortex was separated from medulla, cortical slices were rinsed three times in HBS-Hepes, and placed in a mixture of 5 ml HBS-Hepes, 5 ml of culture medium, 0.25 ml of 10% bovine serum albumin, and collagenase (final concentration = 0.75 mg/ml). This suspension was transferred to a trypsinizing flask, and was incubated under gentle stirring, during 50 to 60 min, at 37° C in a 5% CO_2–95% air atmosphere. After that period, the mixture of renal tubules was washed in HBS-Hepes and centrifuged (200 × *g*; 3 min; 4° C). The pellet was resuspened in the same solution and the operation was repeated three times. The pellet was suspended in 5% albumin HBS-Hepes solution, kept in ice for 5 min, and centrifuged as above. Homogeneous populations of nephron segments were separated by Percoll centrifugation adapted from Vinay et al. [11]. The mixture of tubules was suspended in 50% Percoll made isotonic with 10 × concentrated HBS-Hepes, and was centrifuged (17 000 rpm; 30 min; 4° C) in a Kontron centrifuge equipped with a A8.24 rotor ensuring 14 500–27 000 × *g*. The F_4 layer, made of proximal tubules, was removed, suspended in HBS-Hepes, and was washed and centrifuged (200 × *g*; 2 min; 4° C) three times in this solution. The final pellet was suspended in culture medium and tubules were seeded in 24-well plastic trays ($(2–5) \cdot 10^4$ fragments/well) which had been coated with NH_3-reconstituted rat's tail collagen [12]. Serum-free culture medium consisted in a 1:1 (v/v) mixture of Ham's F-12 and Dulbecco's modified Eagle's medium containing 25 mM Hepes, 21.5 mM HCO_3, 1 mM sodium pyruvate, 10 ml/l of 100 × non-essential amino acid mixture, 4 mM L-glutamine, 50 U/ml penicillin, 50 μg/ml streptomycin, 50 nM sodium selenite , 5 μg/ml insulin, 35 μg/ml transferrin, 20 μM ethanolamine, 5 nM triiodothyronine, and 50 nM dexamethasone. Medium was changed after three days, and then on alternate days. Monolayers of proximal cells reached confluence after 5–6 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 medium, and, on the day of experiment, preincubations with hormones were usually performed in the same medium in which hormones were added as concentrated aliquots.

Uptake studies. Procedures were adapted from Caverzasio et al. [13] and Biber et al. [14] as previously described [7,8]. Briefly, uptakes were performed 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 by replacing sodium chloride with choline chloride. After removal of culture medium, cells were washed with 1 ml/well of the uptake solution, and were incubated for various periods of time in the presence of $K_2H^{32}PO_4$ (0.5 μCi/ml) or [^{14}C]MGP (0.5 μCi/ml) or L-[^{3}H]alanine (1 μCi/ml), and appropriate concentrations of KH_2PO_4, MGP or L-alanine. All these steps were performed at 37° C. At the end on incubation, the uptake was stopped by washing the cells three times with 1 ml/well of ice-cold solution (137 mM NaCl, 15 mM Hepes (pH 7.4). Cells were then solubilized in 0.5% Triton X-100 (250 μl/well) and aliquots were counted by liquid scintillation.

Determination of cAMP content. After removal of culture medium, cells were washed with 1 ml/well of HBS-Hepes, and were then incubated for either 5 or 30 min at 37° C in HBS-Hepes containing 1 mg/ml serum albumin, 0.5 mM IBMX, and hormones (500 μl/well). At the end of incubation, extracellular and intracellular cyclic AMP (cAMP) contents were measured by radioimmunoassays as previously described [4]. When the effect of cholera toxin was studied, cells were preincubated in hormone-free culture medium during three hours in the presence of the toxin at the concentration of 10 μg/ml. The medium was then removed, and cells were incubated during either 5 or 30 min in HBS-Hepes as described above.

Presentation of data. Uptakes of P_i, MGP, and alanine were expressed as nmol/mg protein [15]. Na^+-dependent uptakes were calculated by substracting uptake values measured in the presence of choline from that measured in the presence of Na^+. Cyclic AMP content was expressed as pmol/mg protein and pmol/ml for intracellular and extracellular cAMP, respectively. Results were presented as means ± S.E. of three to five different experiments in which duplicates 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 t-test [16].

Results

In a previous study [7], we have established that similarly cultured cells exhibited several features in favour of their exclusive proximal origin, i.e., (i) existence of Na^+/P_i, Na^+/hexose, and Na^+/alanine cotransports with kinetic parameters similar to those reported in brush-border membrane vesicles; (ii) pattern of hormone-stimulated cAMP synthesis similar to that reported in microdissected proximal tubules [17].

Effect of benzyl alcohol on Na^+-dependent uptakes

The effect of increasing concentrations of benzyl alcohol on the uptakes of P_i, MGP, and alanine is shown in Fig. 1. At low concentrations, up to 10 mM, benzyl alcohol increased P_i uptake, decreased MPG uptake, and did not modify alanine uptake. Benzyl alcohol affected only the Na^+-dependent component of the uptake, whereas the Na^+-independent component was unchanged. At higher concentrations (20–80 mM), benzyl alcohol decreased all three uptakes. However, the magnitude of the inhibitory effect varied from one uptake of the other: at the concentration of 40 mM, benzyl alcohol inhibited by 19%, 86%, and 25% the Na^+-dependent uptake of P_i, MGP, and alanine, respectively (Fig. 1). High concentrations of benzyl alcohol decreased slightly Na^+-independent uptakes as well. The benzyl alcohol-induced changes in the uptakes of P_i and MGP, as well as the lack of effect of the drug on

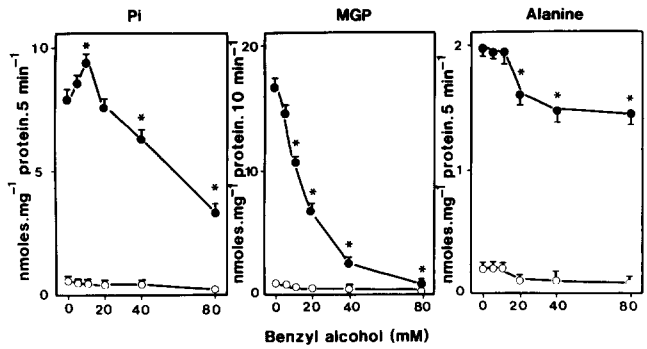

Fig. 1. Effect of increasing concentrations of benzyl alcohol on P_i, MGP, and alanine uptakes. The uptake of P_i (0.1 mM), MPG (1 mM), or alanine (1 mM) were measured in the presence of Na^+ (closed circles) or $choline^+$ (open circles). The data represent the means ± S.E. of four different experiments ($n = 4$) in which duplicates were obtained. * Significantly different from the value without benzyl alcohol.

alanine uptake, were further examined by the kinetic analyses of P_i, MGP, and alanine uptakes in the absence and in the presence of 10 mM benzyl alcohol (Table I). Analysis by the Eadie-Hofstee plot revealed that benzyl alcohol increased the V_{max} of the P_i transport system, decreased the V_{max} of MGP uptake, and did not modify the V_{max} of alanine uptake. Benzyl alcohol had no effect on the K_m values of the three studied transport systems (Table I).

Effect of benzyl alcohol on cAMP accumulation

The stimulatory effect of PTH, forskolin, and cholera toxin on intracellular cAMP accumulation was first determined in the absence of benzyl alcohol. In the absence of IBMX as a phosphodiesterase inhibitor, cAMP content increased significantly from 4.8 ± 0.3 pmol/mg protein per 5 min in the basal state to 18.1 ± 0.5, 50.3 ± 5.7, and 20.1 ± 0.7 pmol/mg protein per 5 min after exposure to PTH, forskolin, and cholera toxin, respectively ($P < 0.01$, $n = 3$). In the presence of 0.5 mM IBMX, intracellular cAMP content increased from 19 ± 2.1 pmol/mg protein per 5 min under basal conditions to 348 ± 44.1, 571 ± 21.1, and 710 ± 19.9 pmol/mg

TABLE I

Effect of benzyl alcohol (10 mM) on the kinetic parameters of Na^+-dependent uptake of P_i, MGP, and alanine

Cells were incubated in the absence (control) or in the presence of 10 mM benzyl alcohol, during 5 min (P_i and alanine uptake) or 10 min (MGP uptake), at 37 °C. K_m and V_{max} values were calculated using the Eadie-Hofstee plot. Results represent the means ± S.E. of four experiments ($n = 4$) in which duplicates were obtained.

	K_m (μM)		V_{max} (nmol/mg protein)	
	control	benzyl alcohol	control	benzyl alcohol
P_i uptake	72 ± 4.4	78 ± 7.9	15.4 ± 0.25	20.1 ± 0.55 [a]
MGP uptake	1966 ± 121.1	2163 ± 167.7	45.0 ± 1.02	34.1 ± 0.98 [a]
Alanine uptake	1158 ± 126.1	1205 ± 42.9	8.5 ± 0.41	8.7 ± 0.26

[a] Significantly different from the value without benzyl alcohol, $P < 0.01$.

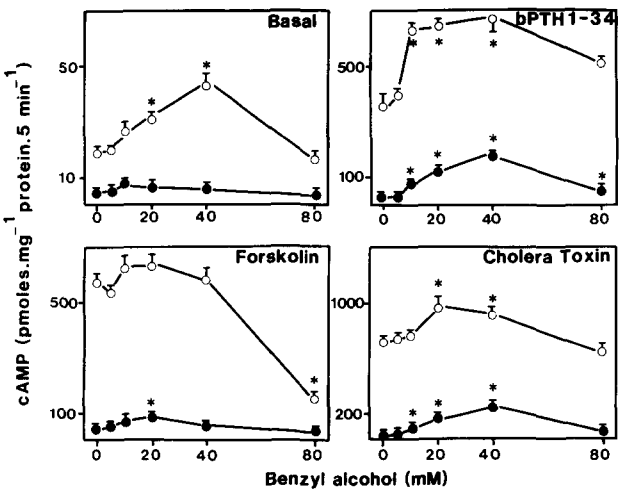

Fig 2. Effect of increasing concentrations of benzyl alcohol on short-term (5 min) intracellular cAMP accumulation. Cells were incubated during a 5-min incubation period, in the absence (closed circles) or in the presence (open circles) of 0.5 mM IBMX. The data represent the means ± S.E. of three different experiments ($n = 3$) in which duplicates were obtained. * Significantly different from the value without benzyl alcohol.

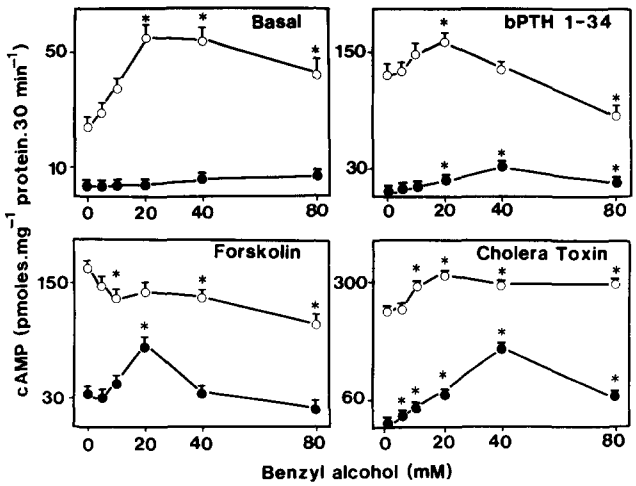

Fig. 3. Effect of increasing concentrations of benzyl alcohol on long-term (30 min) intracellular cAMP accumulation. Cells were incubated during a 30-min incubation period, in the absence (closed circles) or in the presence (open circles) of 0.5 mM IBMX. The data represent the means ± S.E. of four different experiments ($n = 4$) in which duplicates were obtained. * Significantly different from the value without benzyl alcohol.

protein per 5 min after stimulation by PTH, forskolin, and cholera toxin, respectively ($P < 0.001$, $n = 3$).

In the absence of IBMX, benzyl alcohol, up to 40 mM, did not increase basal cAMP level, but increased in a concentration-dependent manner the stimulation elicited by PTH and cholera toxin (Fig. 2, closed circles). At 40 mM, benzyl alcohol increased 7.8-fold and 9.3-fold the stimulatory effect of PTH and cholera toxin, respectively. In contrast, the stimulatory effect of forskolin was only increased 1.7-fold in the presence of 20 mM benzyl alcohol, and was decreased to 0.8-fold the control one in the presence of 40 mM benzyl alcohol (Fig. 2, closed circles). In the presence of IBMX, benzyl alcohol, up to 40 mM, also increased basal, PTH-stimulated and cholera toxin-stimulated cAMP content (Fig. 2, open circles). In contrast, forskolin-stimulated cAMP content was not significantly affected by benzyl alcohol.

Because incubation time with hormones prior to P_i uptake studies was usually 30 min, we also examined the effect of a 30-min incubation period on cAMP accumulation. Increasing the incubation time did not modify the response pattern to hormones and the effect of benzyl alcohol. Again, the stimulatory effect of PTH and cholera toxin on cAMP accumulation was markedly enhanced by benzyl alcohol (Fig. 3).

Effect of benzyl alcohol on PTH-induced inhibition of P_i uptake

A main feature of the proximal tubule is to be the site of PTH-induced regulation of P_i transport, an effect

TABLE II

Modulation of cAMP production and P_i uptake in proximal tubular cells

Cells were incubated in the presence of the indicated concentrations of bPTH 1–34 (PTH), forskolin, IBMX, or dibutyryl-cAMP (dB-cAMP) during 30 min at 37 °C as described in Methods. Cells were then used either for cAMP determination or for P_i (0.1 mM) uptake. Results represent the means ± S.E. of four experiments ($n = 4$) in which duplicates were obtained.

	Intracellular cAMP (pmol/mg protein)	Extracellular cAMP (pmol/ml)	Na^+-dependent P_i uptake (nmol/mg protein per 5 min)
Basal	3.8 ± 0.81	0.5 ± 0.12	7.6 ± 0.24
IBMX (0.5 mM)	14.6 ± 2.53	4.5 ± 0.76 [a]	5.8 ± 0.29 [a]
PTH (0.1 μM)	5.5 ± 0.38	13.7 ± 2.51 [a]	6.1 ± 0.18 [a]
PTH + IBMX	128.0 ± 21.85 [a,b,c]	114.6 ± 14.45 [a,b,c]	4.5 ± 0.30 [a,b,c]
Forskolin (10 μM)	32.3 ± 3.25 [a]	81.7 ± 6.02 [a]	4.8 ± 0.48 [a]
Forskolin + IBMX	153.9 ± 10.64 [a,b]	197.9 ± 9.55 [a,b]	4.2 ± 0.53 [a]
dB-cAMP (100 μM)	181.8 ± 17.39 [a]	n.d.	5.5 ± 0.20 [a]

[a] Significantly different from the homologous basal value, $P < 0.01$.
[b] Significantly different from the value with PTH alone, $P < 0.01$.
[c] Significantly different from the value with IBMX alone, $P < 0.02$.

TABLE III

Effect of benzyl alcohol and of PTH on the kinetic parameters of Na^+-dependent P_i uptake

Cells were preincubated during 30 min at 37 °C in the absence or in the presence of bPTH 1–34 (PTH) and IBMX (0.5 mM) prior to P_i uptake. Benzyl alcohol, at the concentration of 40 mM, was either absent (control) or present in both preincubation and incubation mediums. K_m and V_{max} values were calculated using the Eadie-Hofstee plot. Results represent the means ± S.E. of three different experiments ($n = 3$) in which duplicates were obtained.

	K_m (μM)		V_{max} (nmol/mg protein per 5 min)	
	control	benzyl alcohol	control	benzyl alcohol
Basal	83 ± 8.5	75 ± 8.2	14.5 ± 0.41	10.3 ± 0.30 [a]
PTH (0.1 μM)	89 ± 19.5	81 ± 5.6	12.9 ± 0.78 [b]	7.8 ± 0.15 [a,b]
PTH + IBMX	73 ± 5.2	92 ± 10.6	9.6 ± 0.18 [b,c]	6.5 ± 0.20 [a,b]

[a] Significantly different from the homologous control value, $P < 0.01$.
[b] Significantly different from the homologous basal value, $P < 0.02$.
[c] Significantly different from the value with PTH alone, $P < 0.01$.

thought to be exerted through cAMP production [17]. In our system, we verified that increased cAMP content was associated with decreased P_i uptake (Table II). Accumulation of cAMP induced by IBMX, alone or in combination with PTH or forskolin, occurred concomitantly with a significant decrease of Na^+-dependent P_i uptake. This effect was reproduced by the permeant cAMP analog dibutyryl-cAMP. In contrast, increasing cAMP content with PTH, forskolin or cholera toxin did not influence the uptakes of MGP or alanine (data not shown).

The effect of PTH, associated or not with IBMX, on the kinetic parameters of P_i uptake was studied in the absence or in the presence of benzyl alcohol at 40 mM, a concentration at which PTH-induced cAMP generation was maximally enhanced (Figs. 2 and 3). In the absence of benzyl alcohol, PTH alone had only a modest effect on the V_{max} of P_i uptake, which was decreased by 11 ± 3.6% (Table III), a value which was significantly enhanced in the presence of IBMX. In the presence of benzyl alcohol, the inhibitory effect of PTH alone was more pronounced (24 ± 0.5%, $P < 0.01$ as compared with the value without benzyl alcohol) and the V_{max} of P_i uptake did not further decrease in the presence of IBMX (Table III). Finally, the incubation of P_i uptake induced by PTH in the presence of IBMX was of similar magnitude whether benzyl alcohol was present or not in the medium (34 ± 0.7% vs. 36.7 ± 0.3% in the absence of presence of benzyl alcohol, respectively, not significant) (Table III).

Discussion

The results of the present study indicate that increasing membrane fluidity with the local anesthetic drug benzyl alcohol had dissimilar effects on Na^+/P_i, Na^+/hexose, and Na^+/alanine cotransports, three transport systems located in the apical membrane of proximal tubular cells. Decreasing the viscosity of membrane lipids also enhanced cAMP accumulation elicited by PTH through improved coupling between G_S protein and the catalytic site of adenylate cyclase, as well as PTH-induced inhibition of P_i transport through increased PTH-induced cAMP production.

Dissimilar effect of membrane fluidization by low concentrations of benzyl alcohol on apical cotransports

The benzyl alcohol-induced inhibition of MGP uptake in intact proximal cells is in line with with the results obtained in intact LLC-PK_1 cells [8], an epithelial cell line with proximal features, and with the reported inhibition of Na^+/D-glucose cotransport in renal brush-border membrane vesicles [2]. In the latter work, inhibition of uptake correlated with the increase in fluidity [2]. It is noteworthy that, in all these studies, benzyl alcohol, at 10 mM, inhibited to a similar extent the activity of the transport system. Along the same line, benzyl alcohol was also shown to decrease the number of apparent phlorizin binding sites in renal apical membranes [19]. It seems therefore likely that inhibition exerted by low concentrations of benzyl alcohol was related to a direct effect of the drug on the lipidic environment of the transport system. Three other maneuvers known to increase the fluidity of renal apical membranes, i.e. opening of tight junctions [3,5,6], renal ischemia [19], and vitamin D-3 treatment [20] decreased Na^+-dependent hexose uptake as well [4,7,19,20].

As regards P_i uptake, its stimulation by benzyl alcohol, at concentrations up to 10 mM, resembles that observed in LLC-PK_1 cells and MDCK cells [8]. Similarly, P_i uptake was shown to be enhanced by other maneuvers which decrease the order of membranes lipids, such as opening of tight junctions in both renal epithelial cells lines [8] and in proximal tubular cells [7], and increase in temperature [21], low phosphate diet [22], or administration of vitamin D-3 to vitamin D-depleted rats [23]. In agreement with these results, it was recently shown that superficial renal cortex differed

from juxtamedullary one in that P_i uptake was higher, sphingomyelin content was lower and membranes were more fluid in the former than in the latter [24]. Beyond these correlations, the present results provide direct evidence for an effect of membrane fluidization on P_i transport in the proximal tubule.

The absence of effect of benzyl alcohol at 10 mM on alanine uptake suggests that this transport system in merely sensitive to changes in the physcial state of its lipidic environment. This is consistent with the absence of changes in Na^+-dependent alanine uptake in renal brush-border membranes following ischemia, whereas glucose uptake was sharply decreased [19]. The absence of effect of benzyl alcohol on alanine transport, together with opposite effects of the drug on P_i and MGP uptakes, strongly argues against an action on the Na gradient, resulting either from a modification in membrane permeability to Na^+ or from an alteration of Na^+/K^+-ATPase activity. Such an alteration might have accounted for the inhibitory effect of benzyl alcohol at higher concentrations on all three transport systems, as described in other cells [25].

Mechanism of the effect of benzyl alcohol on cAMP accumulation

That an increase in membrane fluidity modulated the activity of adenylate cyclase and/or cAMP-dependent phosphodiesterase was evidenced in membranes or intact cells originating from several organs such as liver [25,26], heart [27], or kidney [4]. A benzyl alcohol-induced stimulation of phosphodiesterase, such as that reported in liver cell membranes [25], is likely to account for the lack of increase in basal cAMP content in the absence of IBMX, which contrasts with the marked increase in basal cAMP content observed in the presence of IBMX. With respect to the site of action of benzyl alcohol on adenylate cyclase, the stimulation of the enzyme with PTH, cholera toxin, and forskolin allowed to evaluate a possible effect of the fluidizing agent at the level of hormone receptors, regulatory guanine nucleotide-binding proteins, and catalytic subunit, respectively. The following points can be drawn from the results: (i) a predominant effect on PTH receptors is unlikely because benzyl alcohol affected similary the stimulations elicited by PTH and cholera toxin, which ADP-ribosylates directly the stimulatory GTP-binding protein G_S and maintains it in an active form. This is consistent with the result of Martin et al. [9] who reported that, in basolateral membranes of canine renal cortex, benzyl alcohol increased only slightly the number of apparent binding sites of PTH; (ii) that the catalytic subunit is not the major site of action of benzyl alcohol is supported by the lack of stimulatory effect of the drug on cAMP generation induced by forskolin, which is thought to act predominantly at the catalytic site of adenylate cyclase. The inhibitory effect of high concentrations of benzyl alcohol on forskolin-stimulated cAMP generation might have resulted, however, from a competition between benzyl alcohol and forskolin at the adenylate cyclase level [28]; (iii) it appears therefore that benzyl alcohol is likely to facilitate the coupling between G_S and the catalytic subunit. This is in keeping with the reported abolition of the stimulatory effect of benzyl alcohol on renal adenylate cyclase in the presence of 20 mM manganese [9], a maneuver which, among other effects, uncouples G proteins from the catalytic subunit [29,30].

cAMP-mediated modulation by benzyl alcohol of PTH inhibition of P_i uptake

The use of intact proximal tubular cells allowed us to examine the effect of increased cAMP generation on P_i transport. As expected from cells originating from the proximal tubule, maneuvers which increased cAMP content also inhibited P_i uptake [18] (Table II). The magnitude of the effect of PTH alone was modest as compared with that of the hormone in the OK cell line [31,32,33]. This might result from species differences and from the fact that, in rabbit microperfused proximal tubules, PTH was shown to inhibit P_i transport mainly in the straight portion, but not or less in the convoluted one [34,35], a result recently confirmed in rabbit cultured proximal cells [36]. Indeed, the cultured cells used in the present study were likely to originate from both parts of the proximal tubule.

Enhancement of the inhibitory effect of PTH on P_i transport in the present of benzyl alcohol (Table III) is, to our best knowledge, the first experimental evidence that changing membrane fluidity might affect the hormonal modulation of transport function in kidney tissue. Absolute and relative magnitude of PTH-induced inhibition of P_i uptake was larger in the presence than in the absence of benzyl alcohol albeit basal P_i uptake was lower when the fluidizing agent was present. This enhancement resulted most likely from the observed increase in PTH-induced cAMP accumulation under the influence of membrane fluidization. It is noteworthy, however, that the extent to which cAMP content was enhanced did not correlate linearly with the magnitude of inhibition of P_i uptake (Table II). This is due to the marked amplification of the hormonal signal which occurs at the cAMP-dependent protein kinase step, that is, moderate increase in cAMP content is able to elicit maximal stimulation of protein kinase A [37,38]. This probably accounts for the fact that PTH-induced inhibition of P_i uptake was reinforced by benzyl alcohol in the absence of IBMX, when cAMP accumulation was moderate, but not in the presence of IBMX, when cAMP content was already very high.

In conclusion, the present results suggest that: (i) the increase in fluidity of apical membranes of proximal tubular cells induced an increase in P_i uptake, a de-

crease of MGP uptake, and no change in alanine uptake. This reflects differences in the lipidic environments of the transport systems or difference in the sensitivity of these proteins to variations of their lipid environments [2,25]; (ii) the increase in fluidity of basolateral membranes induced an increase in PTH-stimulated cAMP generation through improved coupling between the receptor-G_S complex and the catalytic subunit of adenylate cyclase; (iii) membrane fluidization, through increased cAMP generation, magnified the PTH inhibition of P_i transport; (iv) these variations in the activity of transport and enzymatic proteins may prove to be involved in the modulation of the renal handling of solutes and of the phosphaturic effect of PTH under physiological and pathophysiological conditions.

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References

1 Shinitzky, M. (1984) in Physiology of Membrane Fluidity (Shinitzky, M., ed.,), Vol. 1, pp, 1–51, CRC Press, Boca Raton.
2 Carrière, B. and Le Grimellec C. (1986) Biochim. Biophys. Acta 857, 131–138.
3 Le Grimellec, C., Friedlander, G. and Giocondi, M.C. (1988) News Physiol. Sci. 3, 227–229.
4 Friedlander, G., Le Grimellec, C., Giocondi, M.C. and Amiel, C. (1987) Biochim. Biophys. Acta 903, 341–348.
5 Le Grimellec, C., Friedlander, G. and Giocondi, M.C. (1988) Am. J. Physiol. 255, F22–F32.
6 Van Meer, G. and Simons, K. (1986) EMBO, J. 5, 1455–1464.
7 Friedlander, G. and Amiel, C. (1989) J. Biol. Chem. 264, 3935–3941.
8 Friedlander, G., Shahedi, M., Le Grimellec, C. and Amiel, C. (1988) J. Biol. Chem. 263, 11183–11188.
9 Martin, K.J., McConkey, C.L. Jr. and Stokes, T.J. Jr, (1985) Am. J. Physiol. 248, E31–E35.
10 Bello-Reuss, E. and Weber, M.R. (1986) Am. J. Physiol. 251, F490–F498.
11 Vinay, P., Gougoux, A. and Lemieux, G. (1981) Am. J. Physiol. 241, F403–F411.
12 Steele, R.E., Preston, A.S., Johnson, J.P. and Handler, J.S. (1986) Am. J. Physiol. 251, C136–C139.
13 Caverzasio, J., Brown, C.D.A., Biber, J., Bonjour, J.P. and Murer, H. (1985) Am. J. Physiol. 248, F122–F127.
14 Biber, J., Brown, C.D.A. and Murer, H. (1983) Biochim. Biophys. Acta 735, 325–330.
15 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
16 Snedecor, G.W. and Cochran, W.G. (1972) Statistical Methods, 6th Edn., Iowa University Press, Ames.
17 Morel, F. (1981) Am. J. Physiol. 240, F159–F164.
18 Gmaj, R. and Murer, H. (1986) Physiol. Rev. 66, 36–70.
19 Molitoris, B.A. and Kinne, R. (1987) J. Clin. Invest. 80, 647–654.
20 Elgavish, A., Rikfind, J. and Saktor, B. (1983) J. Membr. Biol. 72, 85–91.
21 Brunette, M.G., Beliveau, R. and Chan, M. (1983) Can. J. Pharmacol. 62, 229–234.
22 Molitoris, B.A., Alfrey, A.C., Harris, R.A. and Simon, F.R. (1985) Am. J. Physiol. 249, F12–F19.
23 Kurnik, B.R.C. and Hruska, K.A. (1984) Am. J. Physiol. 247, F177–F182.
24 Wilson, P., Baird, B. and Levi, M. (1989) Kidney Int. 35, 388A.
25 Gordon, L.M., Sauerheber, R.D., Esgate, J.A., Dipple, I., Marchmont, R.J. and Houslay, M.D. (1980) J. Biol. Chem. 255, 4519–4527.
26 Houslay, M.D. and Gordon, L.M. (1983) Curr. Top. Membr. Transp. 18, 179–231.
27 Roux, S., Escoubet, B., Friedlander, G., Le Grimellec, C., Bertrand, I. and Amiel, C. (1989) Am. J. Physiol. 256, H422-H427.
28 Whetton, A.D., Needham, L., Dodd, N.J.F., Heyworth, C.M. and Houslay, M.D. (1983) Biochem. Pharmacol. 32, 1601–1608.
29 Bellorin-Font, E., Tamayo, J. and Martin, K.J. (1982) Am. J. Physiol. 242, F457–F462.
30 Limbird, L.E., Hickey, A.R. and Lefkowitz, R.J. (1979) J. Biol. Chem. 254, 2677–2683.
31 Caverzasio, J., Rizzoli, R. and Bonjour, J.P. (1986) J. Biol. Chem. 261, 3233–3237.
32 Cole, J.A., Forte, L.R., Eber, S., Thorne, P.K. and Poelling, R.E. (1988) Endocrinology 122, 2981–2989.
33 Malmström, K. and Murer, H. (1986) Am. J. Physiol. 251, C23–C31.
34 Dennis, V.W., Bello-Reuss, E., and Robinson, R.R. (1977) Am. J. Physiol. 233, F29–F38.
35 Brazy, P.C., McKeown, J.W., Harris, R.H. and Dennis, V.W. (1980) Kidney Int. 17, 788–800.
36 Suzuki, M., Capparelli, A., Jo, O.D., Kawaguchi, Y., Ogura, Y., Miyahara, T. and Yanagawa, N. (1988) Kidnet Int. 34, 268–272.
37 Martin, K.J., McKonkey, C.L. Jr. and Garcia, J.C. (1989) Kidney Int. 35, 394A.
38 Onorato, J.J., Middleton, J.P. and Dennis, V.W. (1989) Kidney Int. 35, 180A.