Biochimica et Biophysica Acta 903 (1987) 341–348
Elsevier

BBA 73685

Benzyl alcohol increases membrane fluidity and modulates cyclic AMP synthesis in intact renal epithelial cells

Gérard Friedlander, Christian Le Grimellec, Marie-Cécile Giocondi and Claude Amiel

INSERM U251 and Department of Physiology, Faculté de Médecine Xavier-Bichat, Université Paris 7, Paris (France)

(Received 16 March 1987)

Key words: Membrane fluidity; Forskolin; Glucagon; Vasopressin; cyclic AMP synthesis; Prostaglandin E_2; (MDCK cell)

To evaluate a possible modulation by membrane fluidity of hormonal, cAMP-mediated effects on renal epithelial cells, we studied the effect of the neutral local anesthetic, benzyl alcohol, on membrane fluidity and on basal and stimulated intracellular cAMP content in intact MDCK cells. Benzyl alcohol induced a dose-dependent decrease of lipid order which was measured by steady-state fluorescence anisotropy using trimethylammonium-diphenylhexatriene and propionyl-diphenylhexatriene as fluorescent probes. Benzyl alcohol induced a 2-fold increase in basal cAMP content, likely as a consequence of increased prostaglandin synthesis since this effect was abolished by indomethacin. The effect of benzyl alcohol on stimulated cAMP synthesis depended on the nature of the ligand: 10 mM benzyl alcohol increased significantly the stimulatory effect of prostaglandin E_2, glucagon and forskolin but not of vasopressin. At higher concentrations (40 mM), benzyl alcohol did not affect significantly the glucagon-stimulated cAMP content, while it inhibited significantly the prostaglandin E_2-, forskolin- and vasopressin-stimulated cAMP synthesis. The 40 mM benzyl alcohol-induced inhibition was reversed by 1 mM Mn^{2+}, which is known to block the inhibitory GTP-binding protein N_i. These results suggest that: (i) the various components of the adenylate cyclase-cAMP system and their coupling are affected differently by changes in membrane fluidity, which might reflect differences in their lipid environment, (ii) changes in membrane fluidity can modulate responses of renal tubular cells to hormones, and thus tubular functions.

Introduction

It has been abundantly evidenced that the fluidity of the lipid environment modulates the activity of numerous membrane-bound enzymes, among which adenylate cyclase (for review, see Refs. 1 and 2) [3,4]. Decreasing the order of membrane lipids with local anesthetic drugs [2] was shown to affect dramatically the activity of adenylate cyclase in preparations of plasma membranes isolated from various tissues: the activity was either increased or decreased, depending on the drug which was used [5–9]. Benzyl alcohol, a neutral, water-soluble molecule, was reported to affect differently basal, fluoride-stimulated and hormone-stimulated adenylate cyclase activity in liver plasma membranes [10,11]. Surprisingly, few studies were performed on renal epithelial cells. It was recently shown that benzyl alcohol, up to 100 mM, increased both basal and stimulated adenylate cyclase activity in basolateral plasma mem-

Correspondence: G. Friedlander, INSERM U251, Faculté de Médecine Xavier-Bichat, 16, rue Henri Huchard, F-75018 Paris, France.

branes isolated from dog kidney cortex [12]. However, the effect of benzyl alcohol on intact renal cells has not, to our knowledge, been examined.

The present studies were designed: (i) to examine the effect of increasing concentrations of benzyl alcohol on membrane fluidity and intracellular cAMP accumulation in intact MDCK cells, an established cell line derived from dog kidney cortex which retains differentiated properties of the renal distal tubular epithelium [13,14], (ii) to delineate further the sites of action of benzyl alcohol within the adenylate cyclase complex (hormone receptors, regulatory guanine nucleotide-binding proteins, and catalytic subunit). We show that benzyl alcohol affects cAMP synthesis in a complex manner, resulting from multiple sites of action on the adenylate cyclase-cAMP system.

Materials and Methods

Materials. Indomethacin, insulin, transferrin, hydrocortisone, prostaglandins E_1 and E_2, triiodothyronine, 3-isobutyl-1-methylxanthine and bovine serum albumin were from Sigma (St. Louis, MO). Glucagon was purchased from Novo Industrie (Paris, France), 1-deamino(8-D-arginine) vasopressin (referred to as vasopressin) from Ferring AB (Malmö, Sweden), forskolin from Calbiochem-Behring (La Jolla, CA), and carrier-free $Na^{125}I$ from Amersham (Amersham, U.K.). The two derivatives of diphenylhexatriene: 3-(*p*-(6-phenyl)-1,3,5-hexatrienyl)phenylpropionic acid (propionyl-diphenylhexatriene) and 1-(4-(trimethylamino)phenyl)-6-phenylhexa-1,3,5-triene (trimethylammonium-diphenylhexatriene) were from Molecular Probes Inc. (Junction City, OR), culture media and reagents were from Flow Labs (Irvine, U.K.), and plasticware from Falcon (Oxnard, CA). All other reagents were of analytical grade.

Cell cultures. MDCK cells were obtained from Flow Labs at passage 65. They were used between passages 69 and 75. Cells were seeded in 24-well plastic trays for determination of cAMP accumulation and on microscope glass coverslips (40 mm length, 6 mm width) for fluorescence polarization studies. Cells were grown to confluence at 37 °C, in a 5% CO_2/95% air atmosphere, either in Dulbecco's modified Eagle medium supplemented with 10% fetal calf serum, or in serum-free medium consisting in a 1 : 1 (v/v) mixture of Ham's F-12 and Dulbecco's modified Eagle's medium supplemented with insulin (5 μg/ml), transferrin (5 μg/ml), hydrocortisone (50 nM), triiodothyronine (5 pM) and prostaglandin E_1 (25 ng/ml) as described by Taub et al. [15]. Subcultures were performed once weekly, using a trypsin-EDTA solution, with a splitting ratio of 4 : 1. After 1 month, cultured cells were discarded, and a new culture was initiated from a new batch of cells kept frozen in liquid nitrogen.

Fluorescence polarization studies. Fluorescence polarization measurements were performed [16,17] on a SLM 4800 S apparatus (SLM Inc., Urbana, IL) equipped with a four-cell thermostated compartment and a magnetic stirrer. A temperature-programmable circulator bath was connected to the spectrofluorometer and the temperature was monitored with a thermolinear probe placed directly into the cell. Excitation wave length was 362 nm with a slit width of 4 mm while emission was measured at 430 nm (slit width, 8 mm). Light scattering was reduced to less than 3% by the use of 400-nm cut-off filters placed in the emission light path. Coverslips bearing confluent MDCK cells were washed twice with cold phosphate-buffered saline (125 mM NaCl/20 mM Na_2HPO_4/5 mM NaH_2PO_4/5 mM KCl/0.5 mM $CaCl_2$/2 mM glutamine (pH 7.4)). Using a plastic top, glass coverslips were positioned vertically, 4–5 mm above the bottom of 1 × 1 cm quartz cuvettes filled with 2 ml of phosphate-buffered saline. In order to minimize the amount of exciting light reflected directly into the emission monochromator, the monolayer surface was oriented about 5° from the incident beam. Labeling was achieved by adding, at 37 °C, under gentle stirring, propionyl-diphenylhexatriene or trimethylammonium-diphenylhexatriene (stock solutions: 2 mM in dimethylsulfoxide; final concentration, 0.5 μM) into the fluorometer cuvettes. Using these experimental conditions, the signal over background ratio was higher than 20. In all cases, corrections for stray light and intrinsec fluorescence were made by substracting the values of unlabeled samples from those of identical labeled samples. Results of steady-state depolarization ex-

periments were expressed in terms of fluorescence anisotropy r, with $r = (I_{\parallel} - I_{\perp})/(I_{\parallel} + 2I_{\perp})$, where $I_{\parallel}$ and $I_{\perp}$ were the fluorescence intensities observed with the analysing polarizer parallel and perpendicular to the polarized excitation beam, respectively.

Determinations of intracellular cAMP content. Cells were used when confluent (3–4 days after seeding). After removal of culture medium, the cells were washed twice with Hanks' balanced salt solution (pH 7.4) containing 20 mM Hepes and 1 mg/ml bovine serum albumin (buffer A). Cells were then preincubated for 15 min in buffer A (400 μl/well) containing 0.5 mM isobutylmethylxanthine. After that period, the medium was removed and replaced with 400 μl of fresh buffer A containing 0.5 mM isobutyl methylxanthine, hormones and benzyl alcohol, for a 5 min incubation period. When used, indomethacin, 10 μM, was added to the medium during both preincubation and incubation periods. All these steps were performed at 37 °C. At the end of incubation, the medium was removed and 400 μl of an ice-cold ethanol/formic acid mixture (85 : 15, v/v) were added to each well in order to stop the reaction and to extract cAMP [18]. After 30 min at 4 °C, the ethanol extracts were transferred to glass tubes, evaporated to dryness under a stream of nitrogen, and 500 μl of 50 mM sodium acetate buffer (pH 6.2) were added to each tube. Samples were stored frozen at −20 °C until cAMP radioimmunoassay (RIA) was performed. After acetylation of the samples, cAMP RIA was performed as previously described [19,20]. Intracellular cAMP content was expressed as pmol/well, each well containing 55–60 μg cell protein [21].

Statistical analysis. Results were expressed as mean ± S.E. of three to four (n) separate experiments in which duplicates were obtained. One-way and two-way analyses of variance were performed and, when allowed by the F value, results were compared by the modified t-test [22].

Results

Fluorescence polarization studies

Addition of increasing concentrations of benzyl alcohol to MDCK cells reduced the order of their membrane lipids (Fig. 1). Steady-state anisotropy

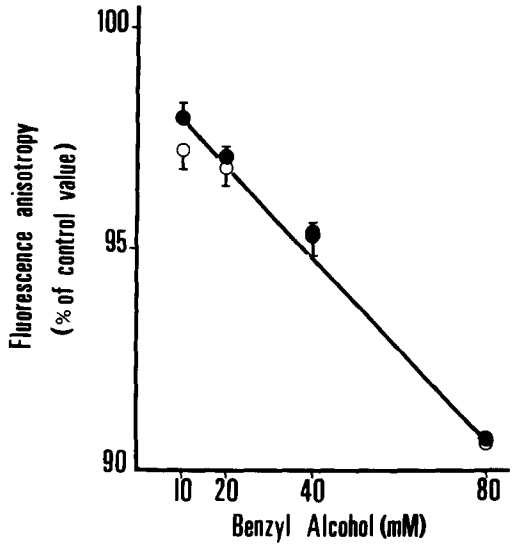

Fig. 1. Effect of increasing concentrations of benzyl alcohol on fluorescence anisotropy measured with trimethylammonium-diphenylhexatriene (●) or propionyl-diphenylhexatriene (○).

of propionyl-diphenylhexatriene in cells maintained at 37 °C decreased from $r = 0.273 \pm 0.003$ ($n = 7$) in controls to $r = 0.246 \pm 0.003$ in the presence of 80 mM benzyl alcohol ($P < 0.001$). A comparable decrease of anisotropy, from $r = 0.320 \pm 0.007$ ($n = 6$) to $r = 0.290 \pm 0.006$ was observed with trimethylammonium-diphenylhexatriene, another diphenylhexatriene analog specifically localised in cell plasma membranes. Similar results were obtained when the two fluorescent probes were used with cells grown in the presence of serum: $r = 0.272 \pm 0.002$ and 0.323 ± 0.007, $n = 6$, for propionyl-diphenylhexatriene and trimethylammonium-diphenylhexatriene, respectively, in control conditions. Also, the response to benzyl alcohol was similar to that of cells grown without serum (not shown).

Effect of benzyl alcohol on intracellular cAMP content

As shown in Table I, vasopressin, glucagon and prostaglandin E_2 increased significantly cAMP accumulation in cells grown in the presence or in the absence of serum. The stimulatory effects of vasopressin and glucagon, used at concentrations which elicited maximal responses, were additive, suggesting that these two hormones stimulate dis-

TABLE I

BASAL AND HORMONE-STIMULATED INTRACELLULAR cAMP CONTENT IN CELLS GROWN IN SERUM-FREE AND SERUM-SUPPLEMENTED MEDIUM

Results are expressed as mean ± S.E. (n = 3). Indomethacin (10 μM) was added to the medium during preincubation and incubation periods. * Significantly different from the homologous control value, $P < 0.01$. ** Significantly different from the value with vasopressin or glucagon alone, $P < 0.01$.

	cAMP (pmol/culture well)	
	Serum-free medium	Medium with 10% serum
Basal	2.2 ± 0.31	10.8 ± 0.69
Basal + indomethacin	1.5 ± 0.25	2.5 ± 0.45 *
Vasopressin (50 nM)	15.3 ± 0.42 *	32.3 ± 6.15 *
Glucagon (1 μM)	12.8 ± 0.46 *	28.3 ± 3.50 *
Vasopressin + glucagon	24.5 ± 0.71 *,**	55.5 ± 4.30 *,**
Prostaglandin E_2 (0.1 μM)	13.0 ± 0.17 *	31.8 ± 3.35 *

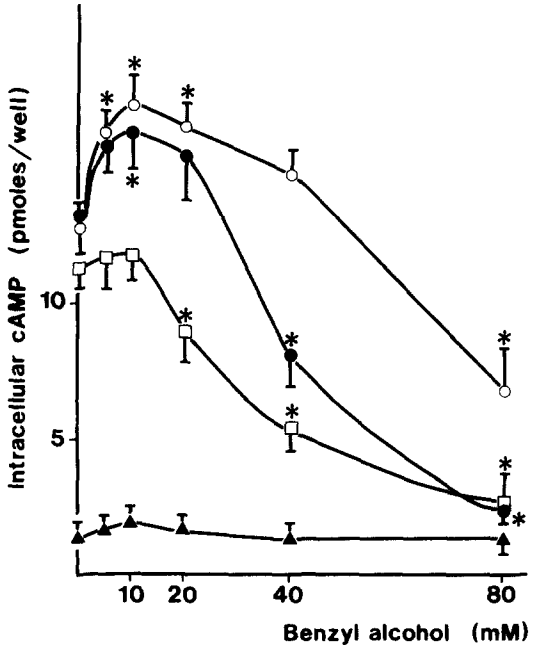

Fig. 2. Effect of increasing concentrations of benzyl alcohol on cAMP content under basal conditions (▲) or during stimulation by 50 nM vasopressin (□), 0.1 μM prostaglandin E_2 (●) and 1 μM glucagon (○). Cells were grown in serum-free medium. * Significantly different from the value without benzyl alcohol, $P < 0.05$.

tinct pools of adenylate cyclase. Basal cAMP content was higher in cells grown in serum-supplemented medium. In the presence of indomethacin, however, basal cAMP content decreased to a value similar to that of cells grown in serum-free medium.

Preliminary experiments had shown that benzyl alcohol, up to 40 mM, increased dose-dependently cAMP content in cells grown in serum-free medium, from 2.2 ± 0.4 pmol/well in controls to 4.7 ± 0.9 pmol/well in the presence of 40 mM benzyl alcohol ($P < 0.05$, $n = 3$). In the presence of 10 μM indomethacin, however, 40 mM benzyl alcohol failed to increase cAMP content, which suggests that part of the effect of benzyl alcohol on cAMP synthesis occurred through stimulation of prostaglandin synthesis [23]. Therefore, all subsequent experiments were performed in the presence of 10 μM indomethacin. In these conditions, the effect of increasing concentrations of benzyl alcohol on basal and hormone-stimulated cAMP accumulation is shown in Fig. 2. At 10 mM, benzyl alcohol increased significantly prostaglandin E_2- and glucagon-stimulated cAMP synthesis by 23 and 33%, respectively, whereas it did not affect significantly basal and vasopressin-stimulated cAMP content. The effect of 40 mM benzyl alcohol was quite different: it decreased the stimulatory effect of vasopressin and prostaglandin E_2 by 51 and 37%, respectively, but not that of glucagon. Finally, 80 mM benzyl alcohol inhibited cAMP content in all stimulated conditions studied. The effect of benzyl alcohol was similar whether cells were grown in serum-supplemented medium (not shown).

The inhibitory effect of benzyl alcohol (40 mM) on vasopressin-stimulated cAMP accumulation occurred at any concentration of vasopressin, as shown in Fig. 3. Benzyl alcohol did not modify significantly the half-maximal stimulation concentration (ED_{50} = 7.8 and 11.7 nM in the absence and presence of benzyl alcohol, respectively).

The possibility that benzyl alcohol might affect adenylate cyclase at sites beyond hormone receptors was evaluated by studying the effect of benzyl alcohol on forskolin-stimulated cAMP generation (Fig. 4). 5 mM benzyl alcohol inhibited by 19 ± 5% ($P < 0.02$) the 0.8 μM forskolin-stimulated cAMP synthesis (right panel). On increasing the con-

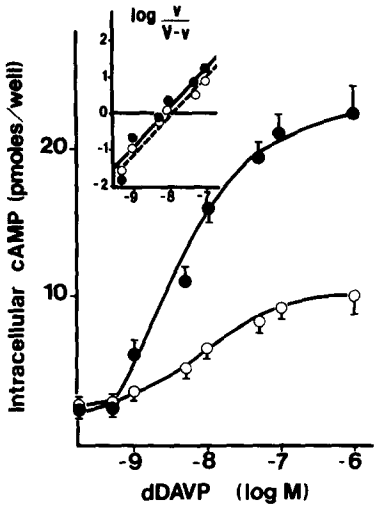

Fig. 3. Effect of increasing concentrations of vasopressin (dDAVP) intracellular cAMP content in the absence (●) or the presence (○) of 40 mM benzyl alcohol. From 1 nM vasopressin, all values were significantly different from the basal value ($P < 0.01$), and all values with benzyl alcohol were significantly different from the homologous ones without benzyl alcohol ($P < 0.01$). Inset: Hill plot.

TABLE II

EFFECT OF Mn^{2+} ON BENZYL ALCOHOL-INDUCED INHIBITION OF INTRACELLULAR cAMP ACCUMULATION

Cells were grown in serum-free medium. Results are expressed as mean ± S.E. ($n = 4$). * Significantly different from the homologous value without $MnCl_2$, $P < 0.05$. ** Significantly different from the control value (without benzyl alcohol), $P < 0.05$.

	$MnCl_2$ (1 mM)	cAMP (pmol/culture well)	
		Control	Benzyl alcohol (40 mM)
Basal	–	1.4±0.12	1.2±0.17
	+	1.6±0.15	1.6±0.20
Vasopressin (50 nM)	–	15.1±0.53	7.2±0.65 **
	+	17.8±0.25 *	12.7±0.75 *,**
Glucagon (1 μM)	–	9.8±1.59	11.0±0.82
	+	23.1±2.96 *	12.9±0.75 **
Prostaglandin E_2 (0.1 μM)	–	12.5±1.30	7.7±0.75 **
	+	18.0±1.05 *	16.7±0.46 *
Forskolin (0.8 μM)	–	26.5±1.66	16.2±0.97 **
	+	33.7±1.85 *	31.8±2.15 *

centration of alcohol, a significant stimulation ensued, followed by an inhibitory effect when the concentration of benzyl alcohol was equal to or higher than 40 mM. As observed with vasopressin, the inhibitory effect of 40 mM benzyl alcohol occurred at any concentration of forskolin and did not modify the ED_{50} value (left panel).

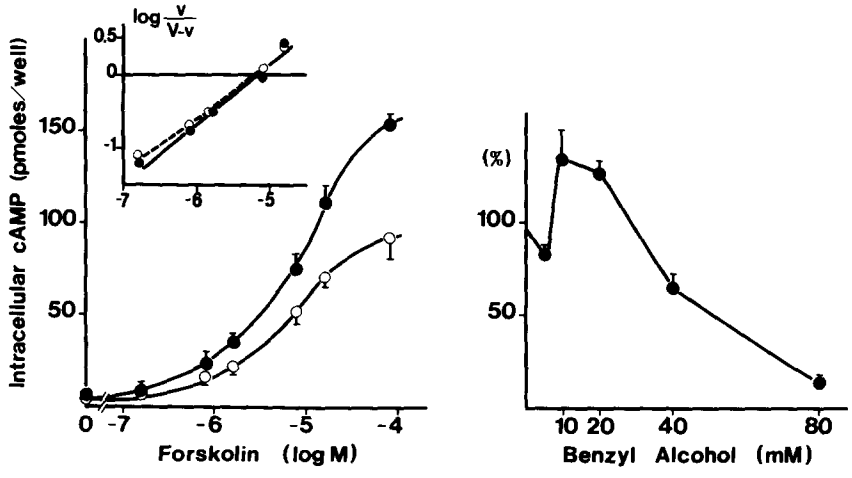

Fig. 4. Left panel: effect of increasing concentrations of forskolin on intracellular cAMP content in the absence (●) or the presence (○) of 40 mM benzyl alcohol. All values with forskolin were significantly different from the basal value ($P < 0.01$), and all values with benzyl alcohol were significantly different from the homologous ones without benzyl alcohol ($P < 0.01$). Inset: Hill plot. Right panel: effect of increasing concentrations of benzyl alcohol on 0.8 μM forskolin-stimulated cAMP content.

In order to evaluate a possible involvement of the inhibitory GTP-binding protein N_i in the action of benzyl alcohol, the effect of benzyl alcohol on stimulated cAMP synthesis was studied while N_i was blocked with 1 mM Mn^{2+}. In the absence of benzyl alcohol, addition of 1 mM $MnCl_2$ to the incubation medium enhanced the stimulatory effect of vasopressin, prostaglandin E_2, forskolin, and, to a greater extent, glucagon on intracellular cAMP content (Table II). 1 mM Mn^{2+} did not modify significantly the effect of benzyl alcohol at low concentrations (up to 20 mM). However, 1 mM Mn^{2+} affected the 40 mM benzyl alcohol-induced changes in cAMP synthesis (Table II) since it reversed partially (vasopressin) or totally (prostaglandin E_2 and forskolin) the inhibition exerted by benzyl alcohol on vasopressin-, prostaglandin E_2- and forskolin-stimulated cAMP generation. On the contrary, 40 mM benzyl alcohol inhibited significantly glucagon-stimulated cAMP synthesis in the presence of 1 mM Mn^{2+}. Finally, Mn^{2+} did not modify the inhibitory effect of 80 mM benzyl alcohol.

Discussion

The present results demonstrate that: (i) the fluidizing effect of the neutral local anesthetic benzyl alcohol on plasma membranes can be evidenced in intact, living renal epithelial cells, (ii) these changes in membrane fluidity affect deeply intracellular cAMP accumulation, likely through effects on various components of the adenylate cyclase-cAMP system.

The effect of benzyl alcohol to increase membrane bilayer fluidity has been documented in membrane preparations from different sources [11,12,24,25], and this effect was shown to be fully and rapidly reversible. Using appropriate fluorescent probes such as trimethylammonium-diphenylhexatriene, it was shown that plasma membrane fluidity can be estimated from fluorescence anisotropy measurements in whole living cells [26,27]. The decrease of fluorescence anisotropy elicited by 40 mM benzyl alcohol in intact MDCK cells was equivalent to that produced by an upward shift in temperature of approx. 6–7 °C, a value within the range of those obtained in other membranes, either isolated [2] or intact cells treated identically [28]. The presence of serum in the culture medium affected neither basal fluorescence anisotropy nor the effect of benzyl alcohol, while it increased cellular prostaglandin synthesis, as evidenced by a higher, indomethacin-inhibited, basal cAMP content in cells grown with serum (Table I). This is in agreement with previous studies, which reported increased contents in arachidonic acid and its essential precursor linoleic acid in membrane phospholipids from MDCK cells when serum was present in the culture medium [29,30]. Taken together, these results suggest that homeoviscous adaptation occurred when cells were grown in serum-deprived medium [31].

As regards the mechanism by which benzyl alcohol affects stimulated cAMP content, it was likely the consequence of a direct action on adenylate cyclase, and could not be accounted for by alterations in the activity of cAMP phosphodiesterase [11] or increased prostaglandin synthesis via alterations of phospholipase A_2 activity [32,33], since the effects of benzyl alcohol were observed in the presence of isobutyl-methylxanthine and indomethacin. The effect of benzyl alcohol was therefore the consequence of an action on one or several components of adenylate cyclase or their coupling. A direct effect at the level of the catalytic subunit is unlikely, since, in the absence of prostaglandin synthesis, basal cAMP content was scarcely affected by benzyl alcohol. This is further supported by previous demonstrations that benzyl alcohol was ineffective to modulate adenylate cyclase activity in detergent-treated plasma membranes [11]. That benzyl alcohol, at 40 mM, enhanced the tonic inhibition exerted by the GTP-binding protein N_i on the enzyme is suggested by the fact that 1 mM Mn^{2+} reversed the inhibition achieved by 40 mM benzyl alcohol on vasopressin-, prostaglandin E_2- and forskolin-stimulated cAMP synthesis. Indeed, the action of low concentrations (less than 5 mM) of Mn^{2+} on adenylate cyclase is thought to be a selective blockade on N_i [34,35], an effect which was also observed on intact renal cells [36,37]. The possibility that Mn^{2+} per se might have exerted an effect on membrane fluidity, such as that described for calcium [38,39], altering in this way responses to hormones, must be considered. However, Mn^{2+} did not modify the effect of low concentrations of

benzyl alcohol, making this hypothesis unlikely. The present study cannot afford a definitive explanation for the stimulatory effect of benzyl alcohol at low concentrations on prostaglandin E_2-, glucagon- and forskolin-stimulated cAMP generation. It can be hypothesized, however, that the stimulation exerted by benzyl alcohol might be the consequence of an improved coupling of N_s with the catalytic subunit, resulting from an increased bilayer fluidity. Indeed, it was reported that, in membranes from renal cortex, uncoupling of the catalytic subunit from the receptor-N_s protein complex achieved by a high (20 mM) Mn^{2+} concentration abolished the stimulatory effect of benzyl alcohol on adenylate cyclase [12,40,41]. An effect of membrane fluidization on the accessibility of membrane receptors cannot be ruled out, and has been reported in other systems [1,42]. It might account for the absence of stimulatory effect of benzyl alcohol on vasopressin-induced cAMP generation. Finally, the strong inhibition achieved by 80 mM benzyl alcohol on ligand-stimulated cAMP accumulation (which returned to the unstimulated value) might be due to benzyl alcohol competing for domains on the protein that are usually occupied by annular lipids [11].

It has recently been reported that ethanol, through a stimulation of phospholipase C activity and of phosphatidylinositol breakdown, released calcium from intracellular stores, thus increasing cytosolic calcium concentration [43]. Such an effect might be due to ethanol-induced fluidization of membranes and, if so, should be shared by other alcohols, such as benzyl alcohol. Therefore, the observed benzyl alcohol-induced inhibition of cAMP synthesis might be interpreted as a calcium-induced inhibition of adenylate cyclase [44]. This hypothesis, however, is made unlikely by the absence of inhibition exerted by 40 mM benzyl alcohol on glucagon-stimulated cAMP content. Following phospholipase C stimulation, generation of diacylglycerol together with the release of intracellular calcium might have led to activate protein kinase C. Evidence of interaction between this system and adenylate cyclase has been provided recently [45,46]. We have previously shown that, in MDCK cells, activators of protein kinase C induced an inhibition of vasopressin-stimulated cAMP synthesis, but did not alter basal cAMP content or its stimulation by prostaglandin E_2, glucagon and forskolin [47]. This makes it unlikely that benzyl alcohol exerted its effects through activation of protein kinase C.

Heterogeneity of the cellular population in MDCK cells has been evidenced by isolation of various clones [48]. In our study, the additivity of the responses to vasopressin and glucagon (Table I) suggests that vasopressin-stimulated and glucagon-stimulated adenylate cyclases do not belong to the same pool, or are located in distinct cell types [49]. That benzyl alcohol did not affect similarly cAMP stimulation by vasopressin and glucagon might suggest differences in the lipidic environments of the two proteic complexes.

In conclusion, the increase in membrane fluidity induced by benzyl alcohol in intact renal epithelial cells modifies the effect of hormones on cAMP synthesis, likely through actions on the various components of the adenylate cyclase-cAMP system and on their couplings. This suggests that the effect of hormones on transporting properties of renal tubules might be modulated by changes in membrane fluidity.

Acknowledgments

The authors acknowledge S. Couette and C. Coureau for technical assistance, and F. Carlier for secretarial assistance.

References

1 Shinitzky, M. (1984) in Physiology of Membrane Fluidity (Shinitzky, M., ed.), Vol. 1, pp. 1–51, CRC Press, Boca Raton
2 Houslay, M.D. and Gordon, L.M. (1983) in Current Topics in Membranes and Transport (Kleinzeller, A. and Martin, B.R., eds.), Vol. 18, pp. 179–231, Academic Press, New York
3 Sinensky, M., Minneman, K.P. and Molinoff, P.B. (1979) J. Biol. Chem. 254, 9135–9141
4 Alam, S.Q., Banerji, A. and Alam, B.S. (1985) Current Eye Res. 4, 1253–1262
5 Houslay, M.D., Dipple, I., Rawal, S., Sauerheber, R.D., Esgate, J.A. and Gordon, L.M. (1980) Biochem. J. 190, 131–137
6 Salesse, R., Garnier, J., Leterrier, F., Daveloose, D. and Viret, J. (1982) Biochemistry 21, 1581–1586
7 Salesse, R., Garnier, J. and Daveloose, D. (1982) Biochemistry 21, 1587–1590

8 Hynie, S., Wenke, M. and Klenerova, V. (1985) Gen. Physiol. Biophys. 4, 517–530
9 Rubalcava, B., Grajales, M.O., Cerbon, J. and Pliego, J.A. (1983) Biochim. Biophys. Acta 759, 243–249
10 Dipple, I. and Houslay, M.D. (1978) Biochem. J. 174, 179–190
11 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
12 Martin, K.J., McConkey, C.L. Jr. and Stokes, T.J. Jr. (1985) Am. J. Physiol. 248, E31–E35
13 Rindler, M.J., Chuman, L.M., Shaffer, L. and Saier, M.H. (1979) J. Cell Biol. 81, 635–648
14 Lin, M.C. and Beckner, K. (1983) in Current Topics in Membranes and Transport (Kleinzeller, A. and Martin, B.R., eds.), Vol. 18, pp. 287–315, Academic Press, New York
15 Taub, M., Chuman, L., Saier, M.H. Jr. and Sato, J. (1979) Proc. Natl. Acad. Sci. USA 76, 3338–3342
16 Le Grimellec, C., Giocondi, M.C., Carrière, B., Carrière, S. and Cardinal, J. (1982) Am. J. Physiol. 242, F246–F253
17 Carmel, G., Rodrigue, F., Carrière, S. and Le Grimellec, C. (1985) Biochim. Biophys. Acta 818, 149–157
18 Chabardès, D., Montegut, M., Imbert-Teboul, M. and Morel, F. (1984) Mol. Cell. Endocrinol. 37, 263–275
19 Brooker, G., Harper, J.F., Terasaki, W.L. and Moylan, R.D. (1979) Adv. Cyclic Nucleotide Res. 10, 2–33
20 Friedlander, G., Chansel, D., Sraer, J., Bens, M. and Ardaillou, R. (1983) Mol. Cell. Endocrinol. 30, 201–214
21 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
22 Snedecor, G.W. and Cochran, W.G. (1972) Statistical Methods, 6th Edn., Iowa State University Press, Ames
23 Hassid, A. (1983) J. Cell. Physiol. 116, 297–302
24 Hutchinson, B.T., Hyslop, P.A., Kuhn, C.E. and Sauerheber, R.D. (1985) Biochem. Pharmacol. 34, 1079–1086
25 Carrière, B. and Le Grimellec, C. (1986) Biochim. Biophys. Acta 857, 131–138
26 Kuhry, J.G., Fonteneau, P., Duportail, G., Maechling, C. and Laustriat, G. (1983) Cell Biophys. 5, 129–140
27 Kuhry, J.G., Duportail, G., Bronner, C. and Laustriat, G. (1985) Biochim. Biophys. Acta 845, 60–67
28 Giraud, F., Claret, M., Bruckdorfer, K.R. and Chailley, B. (1981) Biochim. Biophys. Acta 647, 249–258
29 Lynch, R.D., Locicero, J. and Schneeberger, E.E. (1986) Lipids 21, 447–453
30 Lewis, M.G., Kaduce, T.L. and Spector, A.A. (1981) Prostaglandins 22, 747–760
31 Cossins, A.R. and Sinensky, M. (1984) in Physiology of Membrane Fluidity (Shinitzky, M., ed.), Vol. 2, pp. 1–20, CRC Press, Boca Raton
32 Borochov, H., Zahler, P., Wilbrandt, W. and Shinitzky, M. (1977) Biochim. Biophys. Acta 470, 382–388
33 Casey, L.C., Armstrong, M.C., Fletcher, J.R. and Ramwell, P.W. (1980) Prostaglandins 19, 977–984
34 Cooper, D.M.F., Schlegel, W., Lin, M.C. and Rodbell, M. (1979) J. Biol. Chem. 254, 8927–8931
35 Seamon, K.B. and Daly, J.W. (1982) J. Biol. Chem. 257, 11591–11596
36 Friedlander, G. and Amiel, C. (1986) FEBS Lett. 198, 38–42
37 Teitelbaum, I., Anger, M., Mansour, J. and Berl, T. (1986) Kidney Int. 29, 425A
38 Gordon, L.M., Whetton, A.D., Rawal, S., Esgate, J.A. and Houslay, M.D. (1983) Biochim. Biophys. Acta 729, 104–114
39 Brasitus, T.A. and Dudeja, P.K. (1986) Biochem. J. 239, 625–631
40 Bellorin-Font, E., Tamayo, J. and Martin, K.J. (1982) Am. J. Physiol. 242, F457–F462
41 Limbird, L.E., Hickey, A.R. and Lefkowitz, R.J. (1979) J. Biol. Chem. 254, 2677–2683
42 Dave, J.R. and Witorsch, R.J. (1984) Biochim. Biophys. Acta 772, 321–327
43 Hoek, J.B., Thomas, A.P., Rubin, R. and Rubin, E. (1987) J. Biol. Chem. 262, 682–691
44 Teitelbaum, I. and Berl, T. (1986) J. Clin. Invest. 77, 1574–1583
45 Wakelam, M.J.O., Murphy, G.J., Hruby, V.J. and Houslay, M.D. (1986) Nature 323, 68–70
46 Yoshimasa, T., Sibley, D.R., Bouvier, M., Lefkowitz, R.J. and Caron, M.G. (1987) Nature 327, 67–70
47 Friedlander, G. and Amiel, C. (1987) Biochim. Biophys. Acta 929, 311–317
48 Meier, K.E., Snavely, M.D., Brown, S.L., Brown, J.H. and Insel, P.A. (1983) J. Cell. Biol. 97, 405–415
49 Morel, F., Chabardès, D., Imbert-Teboul, M., Le Bouffant, F., Hus-Citharel, A. and Montegut, M. (1982) Kidney Int. 21, S55–S62