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Modulation by *n*-Alkanols of Rat Cardiac Adenylate Cyclase Activity

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Summary. *n*-Alkanols (from methanol to decanol) have a biphasic effect on rat cardiac adenylate cyclase either basal or stimulated by GTP, GppNHp, NaF or hormones (isoproterenol, glucagon, secretin) in the presence of GTP. At high concentration, all the enzyme activities are inhibited. At low concentration, adenylate cyclase activity is either unchanged or potentiated depending on both the stimulus and the alkanols involved. Potentiation is due to an increase of maximum velocity with no change in the activation constant of the enzyme. Basal activity is unchanged as well as the isoproterenol- and glucagon-stimulated enzyme. The secretin-stimulated enzyme is potentiated. It is the guanyl nucleotide regulatory protein-mediated stimulation of adenylate cyclase which is mainly affected. An attempt was made to relate these effects on adenylate cyclase with physical parameters of the alkanols (partition coefficient). From the data obtained as a function of the alkanol chain-length and of temperature on the adenylate cyclase stimulated by GTP, GppNHp, NaF and permanently activated, it is concluded that the increase in efficacy observed in the presence of alkanol is due to an interaction with the protein moeity particularly with the guanyl nucleotide regulatory protein.

Key Words *n*-alkanols · adenylate cyclase · partition coefficient · rat heart

Introduction

Adenylate cyclase is a multicomponent, membranous enzyme consisting of at least three functional entities: the receptor(s) located at the outer face of plasma membranes, a catalytic unit and guanine-nucleotide regulatory proteins located at the inner face (Ross & Gilman, 1980). The activity of the enzyme is regulated by hormones and guanine nucleotides in a complex cascade of events. As they take place in the membrane, these events are sensitive to the lipidic component of the membrane, as shown by perturbing the lipid matrix *in vitro* (Orly & Schramm, 1975; Rimon et al., 1978; Gordon et al., 1980) and *in vivo* (Engelhard et al., 1976; Sinensky et al., 1979) and/or by changing the temperature (René et al., 1978; Chatelain et al., 1982*b*).

It is widely accepted that the membrane is the site of action of a large variety of molecules. Some of the molecules act through specific proteins (receptors, enzymes) located in the membrane. Other compounds, like anesthetics (including *n*-alkanols), do not have a precise site of action. Their interactions with the membrane appear in part to be physical in nature since a relationship between the anesthetic potency of a compound and its lipid solubility has been established (Seeman, 1972). This observation initiated numerous studies on the effects of anesthetics (and *n*-alkanols) on the dynamics of lipids. These effects included membrane expansion (Seeman, 1972), lipid disorder (Chin & Goldstein, 1977; Lyon et al., 1981) or lipid phase transition (Paterson et al., 1972; Lee, 1976; Jain & Wu, 1977). These effects have been related to some extent to the variation of activity of membranous enzymes like brush-border Na^+, K^+-ATPase and Mg^{2+}-ATPase (Mitjavila et al., 1976), galactoside transport in *Escherichia coli* (Jain et al., 1978), phospholipase A_2 (Jain et al., 1978) or adenylate cyclase (Stock & Schmidt, 1978). However, the exact implications of the effects on lipid dynamics for protein structures and functions are still questioned and a direct effect on proteins is certainly not excluded (Franks & Lieb, 1982).

In this communication, the effects of a homologous series of aliphatic alcohols ranging from methanol to decanol was systematically investigated on basal and stimulated rat cardiac adenylate cyclase at 37°C and as a function of temperature. The stimulation of adenylate cyclase was achieved either by guanyl nucleotide (GTP, GppNHp) and NaF acting through the guanine-nucleotide regulatory protein (Ross & Gilman, 1980) or by isoproterenol, glucagon and secretin acting, in the presence of guanyl nucleotides, through three distinct receptors (Chatelain et al., 1980).

Materials and Methods

Chemicals

n-Alkanols (methanol, ethanol, propanol, butanol, pentanol, hexanol, heptanol, octanol, decanol) and synthetic secretin were purchased from Fluka (Buchs, Switzerland). Alkanols were of purist (>99%) quality. GTP[1], ATP grade 1 and (±) isoproterenol were obtained from Sigma Chemical Company (St. Louis, MO). GppNHp and GTP γs were from Boehringer (Mannheim, Federal Republic of Germany). Porcine glucagon was provided by Novo Industrie (Ets Couvreur, Brussels, Belgium). [α^{32}P] ATP (New England Nuclear, Dreiech, Federal Republic of Germany) and cyclic [8-^{3}H] AMP (the Radio-Chemical Centre, Amersham, Bucks, England) had specific radioactivity of 10 to 20 and 27 Ci/mmol, respectively.

Preparation of Cardiac Membranes

The procedure of Snyder and Drummond (1978) described for rabbit heart, was adapted to rat tissue with few modifications (Chatelain et al., 1980). The heart was dissected out, rinsed at room temperature with isotonic sodium chloride, and minced with scissors. All subsequent operations were performed at 4°C. The tissue was suspended in 20 ml of 20 mM Tris-HCl containing 2 mM dithioerythritol and 5 mM $MgCl_2$ (pH 7.5), and homogenized with a glass-Teflon® homogenizer. After a twofold dilution with the same buffer, the homogenate was filtered through two layers of medical gauze and centrifuged at 520 × *g* for 10 min. The pelleted membranes were washed once with 10 ml of the homogenization buffer and resuspended in 5 ml of 20 mM Tris-HCl buffer (pH 7.5) containing 0.25 M sucrose and 5 mM $MgCl_2$. An equal volume of 20 mM Tris-HCl buffer (pH 7.5) enriched with 0.25 M sucrose, 2 mM dithioerythritol, and 2.5 M KCl was added dropwise. The final mixture was stirred for 1 hr in a cold room. The final suspension was centrifuged at 37,000 × *g* for 10 min and the resulting pellet was washed three times with a 20-mM Tris-HCl buffer (pH 7.5) enriched with 0.25 M sucrose and 2 mM dithioerythritol. The membranes were finally resuspended in an adequate volume of the last buffer in order to obtain a concentration of approximately 6 mg of protein per milliliter. Protein was assayed by the method of Lowry et al. (1951).

Adenylate Cyclase Assay

Adenylate cyclase activity was determined by the conversion of [α-^{32}P] ATP into cyclic [^{32}P] AMP. The standard assay medium contained the following final concentrations: 0.5 mM ATP including 10^6 cpm [α-^{32}P] ATP per assay, 5 mM $MgCl_2$, 0.5 mM ethylene glycol bis (β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid, 1 mM cyclic AMP, 1 mM theophylline, 30 mM Tris-HCl, and an ATP-regenerating system that consisted of 10 mM phospho (enol) pyruvate and pyruvate kinase (30 μg/ml). The pH of the final medium was adjusted to 7.5 at 37°C. After 8 min of incubation, the reaction was stopped by addition of 0.5 ml of 0.5% sodium dodecyl sulfate solution containing 1.5 mM ATP, 0.5 mM cyclic AMP, and cyclic [8-^{3}H] AMP (20,000 cpm in order to determine nucleotide recovery). Cyclic AMP was separated from ATP by two successive chromatographies (Salomon et al., 1974). Under our experimental conditions, the kinetics was found to be linear and was not affected by the moderate increase in the pH of the incubation medium at lower temperatures (from 7.5 at 37°C to 7.7 at 17°C) (Chatelain et al., 1982*a*,*b*).

Persistent activation of rat cardiac adenylate cyclase was achieved by incubating 4 to 5 mg membrane protein at 30°C with saturating concentrations of GppNHp and isoproterenol in a 20-mM Tris-HCl, 0.25 M sucrose, 2 mM dithioerythritol buffer (pH 7.5). After 5 min, the reaction was stopped by addition of a large excess of the same ice-cold buffer. The suspension was immediately centrifuged at 37,000 × *g* for 10 min at 4°C. The pellet was rehomogenized in the same buffer and centrifuged again. The washing procedure was repeated three times. Washed membranes were finally resuspended in the same buffer and processed in the same way as the untreated membranes. When tested, the high adenylate cyclase activity was no longer enhanced by addition of hormones alone or in combination with guanyl nucleotides and was therefore considered as maximal.

Calculation of Aqueous Concentration, Partition Coefficient, Membranous Concentration and Membranous Volume of Alkanols

In order to take into account the actual aqueous concentration of alkanols in further calculations, the aqueous concentration was calculated as already described (Lyon et al., 1981). The total added concentration was distributed as follows:

$$C_t = \frac{C_a V_a + C_m V_m}{V_t} \tag{1}$$

where C is concentration, V is volume and subscripts, a, m and t represent aqueous, membrane and total, respectively. Defining the partition coefficient $P_{\text{memb}} = C_m/C_a$ and solving for C_a,

$$C_a = \frac{C_t V_t}{V_a + V_m P_m}. \tag{2}$$

Our membrane preparations contained 5 mg/ml of protein. Estimating equal amounts by weight of lipid and protein and overall membrane density of 1, Eq. (2) reduces to

$$C_a = \frac{C_t}{0.99 + 0.01 P_m}. \tag{3}$$

The membrane/buffer partition coefficient, P_m (moles per kilogram of membrane/moles per liter of water), of each alkanol was calculated from the octanol/water partition coefficient, P_{oct} (Leo et al., 1971) divided by 5 since Roth and Seeman (1972) demonstrated that P_m *for n*-alcohols in erythrocyte and neuronal membrane is equal to one-fifth of P_{oct}. The membranous concentration (mmol per gram of membrane) of alkanol was obtained by multiplying the aqueous concentration C_a by the membrane/buffer partition coefficient P_m. The membranous volume was estimated by multiplying the membranous concentration by the molecular volume of the corresponding alkanol. The molecular volume was calculated by the method of Bondi (1964).

[1] *Abbreviations:* ATP, adenosine triphosphate; cyclic AMP, adenosine monophosphate cyclic; GTP, guanosine triphosphate; GppNHp, guanosine 5′-0-(β, γ,-imido) triphosphate; E_a, energy of activation; Sn, secretin

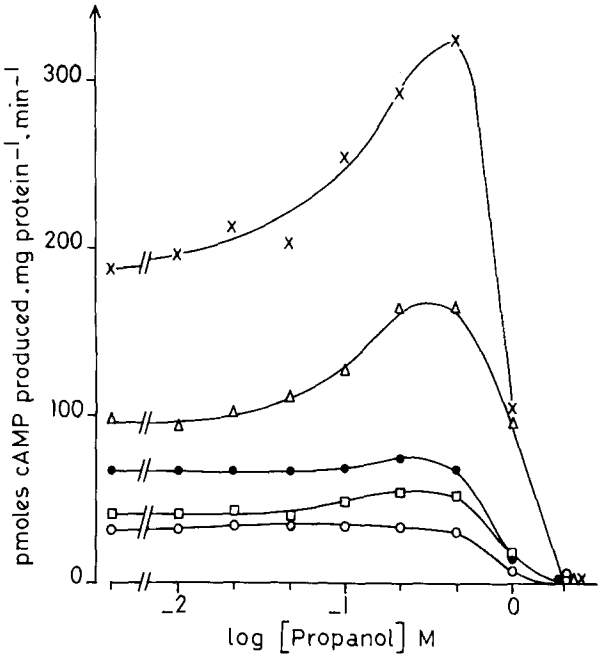

Fig. 1. Dose-response curve of the effect of *n*-propanol on the basal and stimulated rat cardiac adenylate cyclase. Conditions tested were: (○) basal, (□) 10^{-5} M GTP, (●) 10^{-5} M isoproterenol + 10^{-5} M GTP, (△) 10^{-5} M GppNHp, (×) 10^{-2} NaF. Adenylate cyclase activity is expressed as pmol cAMP produced · min^{-1} · mg protein^{-1}. The data represent the result of a typical experiment performed in duplicate and representative of three others

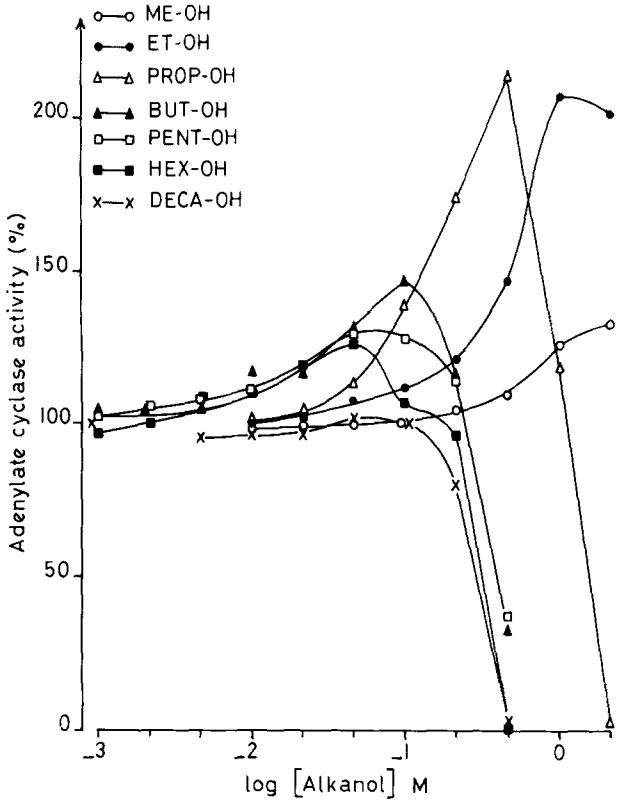

Fig. 2. Comparative effects of *n*-alkanols on the GppNHp-stimulated rat cardiac adenylate cyclase. The following alkanols are compared: ① ○ methanol, ② ● ethanol, ③ △ propanol, ④ ▲ butanol, ⑤ □ pentanol, ⑥ ■ hexanol, ⑩ × decanol. The GppNHp concentration is 10^{-4} M which yields an adenylate cyclase activity of 95 pmol cAMP produced · min^{-1} · mg protein^{-1}. For comparison, results are expressed as percent of this value. They represent the mean of 3 to 4 experiments performed in duplicate. Standard error of the mean never exceeds 10%

Results

A typical profile of the various effects of increasing concentrations of *n*-alkanols on the activity of rat cardiac adenylate cyclase is shown in Fig. 1 where *n*-propanol was used. Up to 0.6 M, *n*-propanol has no effect on the basal, a mild stimulating effect on the isoproterenol-activated enzyme and markedly increases the enzyme activity in the presence of GTP, GppNHp and NaF. At higher concentration, the adenylate cyclase activity decreases abruptly in all conditions tested. The increase in the adenylate cyclase amounts maximally to between 40 and 100% depending on the stimulus. Qualitatively similar results were observed when the rat cardiac adenylate cyclase was incubated in the presence of the other alkanols used in this study (*data not shown*). Due to the marked effect of the *n*-alkanols on the sodium fluoride- and GppNHp-stimulated enzyme, the activation process occurring at the level of the guanyl nucleotide regulatory protein was particularly investigated.

Figure 2 compares the effects of a series of alkanols on the rat cardiac adenylate cyclase activity stimulated by 10^{-4} M GppNHp. Results are presented in percent of the adenylate cyclase activity obtained in the absence of alkanols. All the alkanols tested have a biphasic action upon the enzyme activity. However, both the maximum level of stimulation and the concentration of alkanol required to attain this maximum level vary. These variations are observed mainly for the alcohols containing one to five carbon atoms. Hexanol and heptanol activate the enzyme slightly and in the same manner at the same low aqueous concentration. Octanol activates the enzyme by 5 to 10% at the same aqueous concentration. Decanol does not activate rat cardiac adenylate cyclase but inhibits the enzyme activity above 10^{-1} M. As exemplified in Fig. 3, for both ethanol and propanol at their maximal stimulatory effect, the alkanols do not alter the configuration of the dose-response curve of GppNHp (Fig. 3*A*) and NaF (Fig. 3*B*), indicating that the alkanols increase the efficacy but not the potency of the stimulus tested.

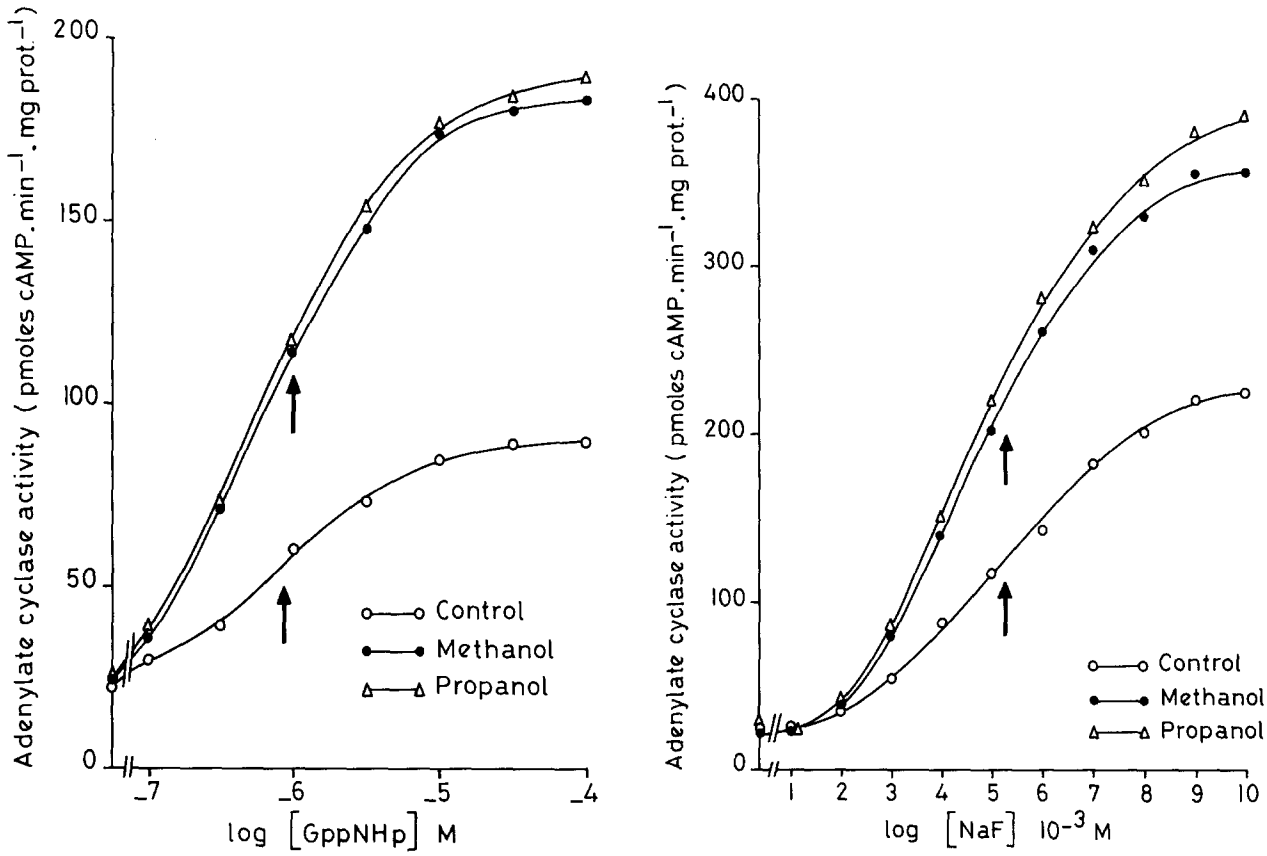

Fig. 3. Dose-response curve of activation of rat cardiac adenylate cyclase by GppNHp (*A*) and NaF (*B*). The stimuli are tested alone (○) and in the presence of 1 M methanol (●) or 0.4 M propanol (△). Adenylate cyclase activity is expressed as pmol cAMP produced · min^{-1} · mg $protein^{-1}$ and represents the mean of three experiments performed in duplicate. Standard error of the mean never exceeds 10%

Table 1. Total concentration at the maximal stimulation and maximal stimulation of the adenylate cyclase activity mediated by the guanine-nucleotide regulatory protein

Alkanol	*C* (M)	Percentage of activation in the presence of			
		GTP (10^{-4} M)	GppNHp (10^{-4} M)	NaF (10^{-2} M)	(GppNHp) persis.
Methanol	2	0	31	27	16
Ethanol	1	16	107	69	41
Propanol	0.4	44	111	85	55
Butanol	0.15	37	73	44	45
Pentanol	0.08	26	59	30	21
Hexanol	0.04	5	25	0	4
Heptanol	0.04	0	19	0	0
Octanol	0.06	0	16	0	0
Decanol	—	0	0	0	0

A similar pattern of activation of the enzyme at low concentrations and at high concentrations of *n*-alkanols is observed when the adenylate cyclase is permanently activated (Fig. 4). In this state, the catalytic unit of the adenylate cyclase and the guanyl nucleotide regulatory protein are thought to be permanently coupled. Therefore, the activity is considered as maximal, as in the absence of alkanol, the addition of a guanyl nucleotide or a hormone (in the absence or in the presence of a guanyl nucleotide) does, in fact, fail to increase cAMP production any further.

The various effects of alkanols on the guanyl nucleotide regulatory protein-activated adenylate cyclase are compared in Table 1. Firstly, for all the conditions tested, the maximal effect of the alkanols is achieved for the same aqueous concentration. Secondly, for all the conditions tested, the stimulatory effects of *n*-alkanols are qualitatively the same, propanol being the most efficient. The effect increases from methanol to propanol and then decreases with the lengthening of the hydrocarbon chain. Thirdly, for a given alcohol, the maximal effect is observed according to the following sequence of activator:

GppNHp > NaF > GppNHp persis. > GTP.

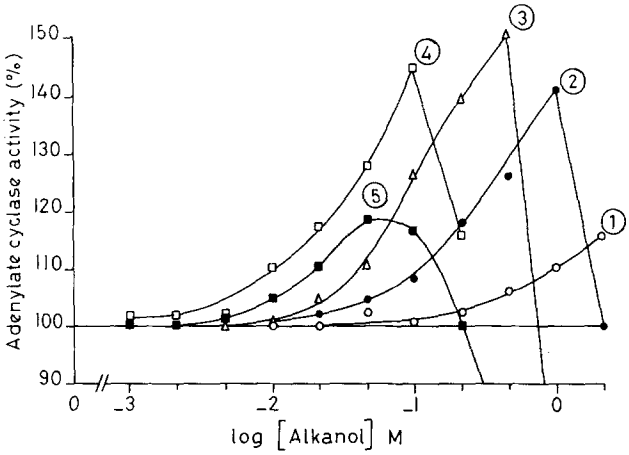

Fig. 4. Comparative effects of *n*-alkanols on the permanently activated rat cardiac adenylate cyclase. The following alkanols are compared: ① ○ methanol, ② ● ethanol, ③ △ propanol, ④ □ butanol, ⑤ ■ pentanol. Permanent activation of adenylate cyclase was achieved as described in Materials and Methods. Enzyme activity is of ≅ 270 pmol cAMP produced · min^{-1} · mg $protein^{-1}$ and cannot be further increased by guanyl nucleotide(s) alone or in the presence of a hormone. For comparison, results are expressed as percent of this value. They represent the mean of three experiments performed in duplicate. Standard error of the mean never exceeds 10%

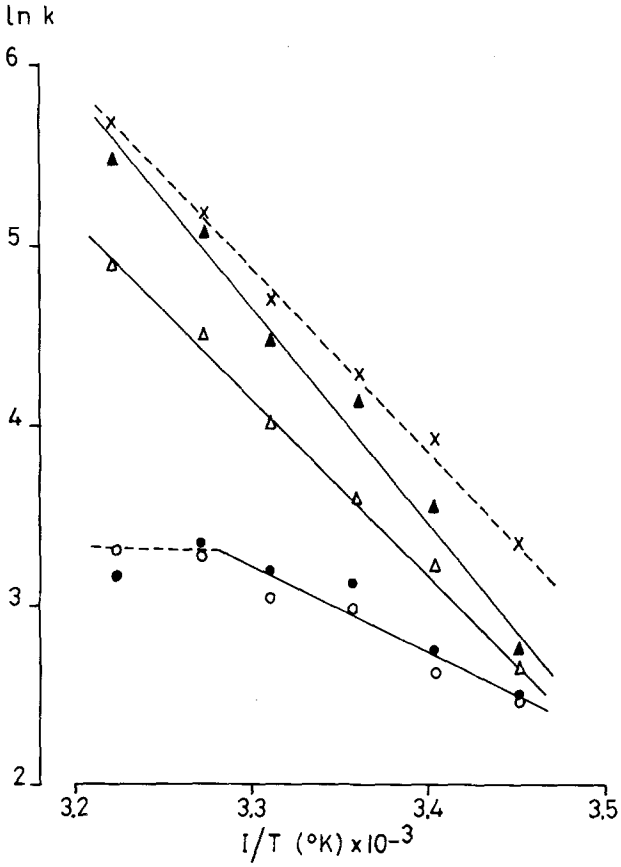

Fig. 5. Temperature dependence of rat cardiac adenylate cyclase stimulated by 10^{-5} M GTP (○, ●) and 10^{-4} M GppNHp (△, ▲) in the absence (open symbols) and in the presence (closed symbols) of 0.4% propanol. The dotted line represents data corrected for the maximal stimulatory effect of *n*-propanol. The initial rate-constant of cAMP production (K_r) is expressed as pmol cAMP produced · min^{-1} · mg $protein^{-1}$. Results represent the mean of three experiments performed in duplicate. Standard error of the mean never exceeds 10%

To further characterize the effect of alkanols on the stimulation of adenylate cyclase, the enzyme activity was examined as a function of temperature for the basal and the GTP-, GppNHp- and NaF-stimulated enzyme. Arrhenius representations (Fig. 5) of the GTP- and GppNHp-stimulated enzyme in the absence and in the presence of 0.4 M propanol indicate that the alcohol has no effect on enzyme activity in the presence of GTP at all temperatures. The same observation was made in the basal condition (*not shown*). This is in agreement with and expands on the observation performed at 37°C (Fig. 1). It is interesting to note the absence of change in the slopes, i.e., in the energies of activation (E_a) of the process of activation of the enzyme and the absence on effect of the break-point around 31°C. The latter has been associated with a lipid phase transition in the inner face of rat heart plasma membranes due to the coincidence of the high temperature onset (32°C) of a thermotropic lipid phase transition demonstrated by ESR technique (Gordon et al., 1978) and the break in basal and GTP-stimulated adenylate cyclase (Chatelain et al., 1982*b*, 1983). In the presence of GppNHp (Fig. 5) and NaF (*not shown*), the Arrhenius plot is linear both in the absence and in the presence of 0.4 M propanol. The alcohol has the effect of increasing the energy of activation (E_a) of the process of activation of the enzyme. For membranous enzyme, Arrhenius plots are thought to represent, at least in part, the interaction between the lipid matrix and the proteins (Sandermann, 1978). Thus, an increase in E_a could reflect a decrease in fluidity (or mobility) of the lipids which is surprising in view of the well-known "fluidizing" effect of the lipid matrix produced by alkanols (Paterson et al., 1972; Lee, 1976; Jain & Wu, 1977; Chin & Goldstein, 1977, Lyon et al., 1981). This apparent contradiction can be removed by careful examination of the effect of alkanols at various temperatures (Fig. 6). For the two alcohols chosen as examples, decreasing the temperature of incubation from 37 to 25°C displaces the maximal effect to a higher concentration but without changing the maximal effect (when expressed in percent of the control value). This result is expected from the known dependence of the partition coefficient as a function of temperature (Diamond & Katz,

Table 2. Aqueous concentrations, membranous concentrations and membranous volumes of alkanols increasing by 20% the activity of rat cardiac adenylate cyclase stimulated by GppNHp 10^{-4} M[a]

Alkanol	C_a[b] (M)	P_m $\left(\frac{\text{mol/kg membr}}{\text{mol/liter water}}\right)$	Membr. Conc $\left(\frac{\text{mmol}}{\text{g membr}}\right)$	Mol. Vol $\left(\frac{\mu\text{l}}{\text{mmol}}\right)$	Membr. Vol $\left(\frac{\mu\text{l}}{\text{g membr}}\right)$
Methanol	0.6	0.04	0.024	21.8	0.52
Ethanol	0.2	0.10	0.020	31.9	0.64
Propanol	0.05	0.44	0.022	42.2	0.93
Butanol	0.03	1.52	0.045	52.4	2.36
Pentanol	0.019	5.02	0.096	62.6	6.01
Hexanol	0.025	21.4	0.533	72.9	38.9
Heptanol	0.060	66.2	3.97	83.6	31.9

[a] The calculation of the other parameters is described in Materials and Methods.
[b] Obtained from Fig. 2.

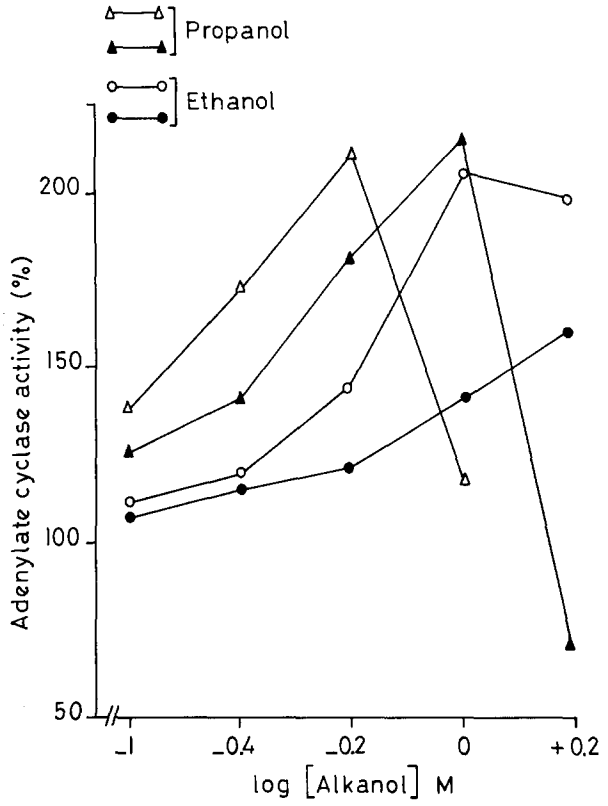

Fig. 6. Dose-response curve of the effect of propanol (△, ▲) and ethanol (○, ●) on the rat cardiac adenylate cyclase stimulated by 10^{-4} M GppNHp at two temperatures, 37°C (open symbols) and 25°C (closed symbols)

1974). Taking this information into account, it can be shown that the actual effect of propanol at 25°C corresponds to the membranous concentration of the alcohol which decreases with temperature and the expected maximal effect of propanol can be calculated. Thus, the increase in E_a is due to a decrease in the effect of propanol resulting from a decrease in its membranous concentration. Since the maximum stimulating effect of propanol is unchanged, the Arrhenius plot, as shown by the dotted line in Fig. 5, is parallel to that obtained in the absence of alcohol. This thus suggests the absence of effect of alcohol on the rat heart adenylate cyclase activity mediated by an effect on the lipid matrix, although at the same range of concentration, *n*-propanol has a well-defined effect on lipid dynamics (Paterson et al., 1972; Lee, 1976; Jain & Wu, 1977; Lyon et al., 1981).

As the nature of the membrane-alkanols interaction is thought to be similar for each alkanol (*see* Introduction), we postulate that the same theoretical maximal activity should be obtained and that parallel dose-response curves should be developed. Based on this assumption, we compared the alkanol concentrations required to equally stimulate adenylate cyclase. The situation is, however, complicated by the fact that the relative potencies of either activation or inhibition of adenylate cyclase do not have the same ratios so that the observed (activation-inhibition) dose-response curves differ from one alkanol to another. Table 2 shows the aqueous alcohol concentrations required to produce an increase of 20% in the activity of the GppNHp-stimulated adenylate cyclase derived from Fig. 2. From their aqueous concentration and partition data, the membranous alcohol concentration is calculated as described in Materials and Methods. The membranous concentration producing a 20% increase in enzyme activity is constant for the first three alcohols only. It then increases rapidly with the increase of chain-length to reach 165 times the initial value. In the meantime, the aqueous concentration varies by a factor of 30. The alcohol membranous volumes responsible for a 20% increase in enzyme activity were also estimated from the membranous concentration and the molecular volume. Contrary to the

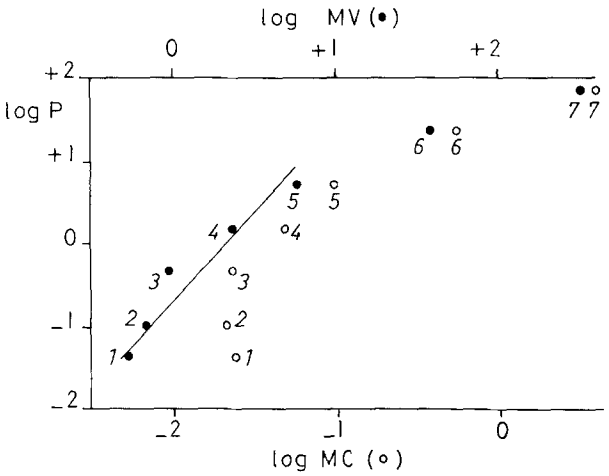

Fig. 7. Relationship between the partition coefficient (log P) of *n*-alkanols with their membranous concentration (log MC, ○) and the corresponding membranous volume (log MV, ●). Data are from Table 2. Numbers (1 to 7) represent the number of carbon atoms in each alkanol

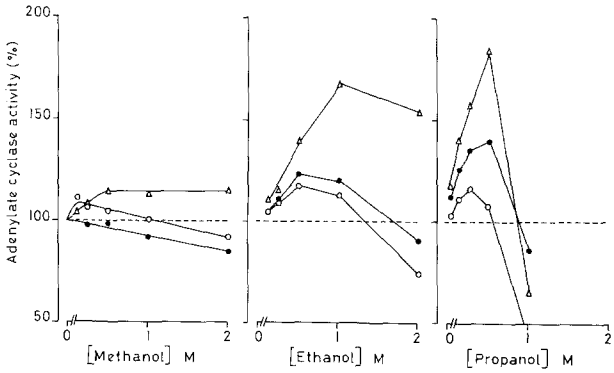

Fig. 8. Dose-response curve of the effect of methanol (left panel), ethanol (middle panel) and propanol (right panel) on rat cardiac adenylate cyclase, stimulated by 10^{-4} M isoproterenol (○), 10^{-6} M glucagon (●) and 10^{-6} M secretin (△). Hormones are tested in the presence of 10^{-5} M GTP. For comparison, results are as percent of the activity in the presence of 10^{-5} M GTP. They represent the mean of 3 to 4 experiments performed in duplicate. Standard error of the mean never exceeds 10%

membranous concentration, the membranous volume increases continuously from methanol to heptanol. The evolution of both the membranous concentration and membranous volume of alkanols producing the same 20% increase in adenylate cyclase activity as a function of their partition coefficient (*P*) (Fig. 7) illustrates that none of the two former parameters is constant with *P* with the exception of the membranous concentration of methanol, ethanol and propanol. Moreover, there appears a linear relationship ($y = 1.87x - 0.62$, $r = 0.96$) between the log of the membranous volume occupied by the first five alkanols and the log of *P* which suggests the importance of other factors in addition to *P*.

The rat cardiac adenylate cyclase can be stimulated by three distinct classes of receptors recognizing the β-adrenergic agonist, the glucagon and the secretin-vasoactive intestinal peptide (VIP), respectively. Although alkanols activate the isoproterenol-stimulated enzyme only slightly (Fig. 1), their effects were examined on the glucagon- and secretin-stimulated enzyme. Figure 8 compares the effects of the first three alcohols on the isoproterenol-, glucagon- and secretin-activated enzyme. For these three alkanols, the stimulatory effects on the isoproterenol- and glucagon-stimulated enzyme are lower than the effect obtained in the presence of GTP alone (Fig. 1, Table 1). In the case of secretin, the stimulation mediated by the hormone is potentiated by the alkanol. The increase in enzyme activity occurs with no change in the potency of secretin as observed from the complete dose response curve (*data not shown*). Note that the alkanol aqueous concentration at the maximal effects corresponds to that previously observed at the level of the guanyl nucleotide regulatory protein (Fig. 1, Table 1).

Discussion

The above data indicate that a homologous series of *n*-alkanols, ranging from methanol to decanol, has numerous effects on the rat heart adenylate cyclase. As a function of their aqueous concentrations, the *n*-alkanols have two clearly distinct effects. At low concentrations, they have either no effect or they increase the enzyme activity. These effects depend on both the alkanol chain-length and the level of adenylate cyclase activity. This will be discussed further on. At high concentrations, whatever the experimental conditions, a sharp decrease in enzyme activity is observed. This can be interpreted as protein denaturation in accordance with the known power of organic solvents to disrupt hydrophobic interactions between the nonpolar side-chains of amino acids (Tanford, 1968). In our experimental conditions, this might reflect essentially the denaturation of the catalytic unit of the enzyme. This conclusion has been directly tested by washing out the alkanol and assaying the basal and GppNHp-stimulated enzyme activity. Since the activity does not return to the levels observed in the absence of alkanol, we can conclude to an irreversible denaturation.

At low concentrations, the *n*-alkanols do not modify basal activity. This is in partial agreement with data obtained with a limited series of alkanols

in plasma membranes of rat adipocyte (Stock & Schmidt, 1978). In this model, methanol and ethanol had no significant effect but butanol and propanol increased basal activity twofold. If we consider that basal activity reflects the activity of the catalytic unit, the lack of activation-effect of *n*-alkanols could be rationalized in the light of recent data obtained on the interactions between the catalytic unit and lipids (Ross, 1982; Stengel et al., 1982) indicating that the catalytic unit is probably not embedded in the lipid matrix except for a small part where the interaction with the lipids takes place (Ross, 1982) and which is essential for coupling with the guanyl nucleotide regulatory protein (Stengel et al., 1982). In this configuration, the possible effects of *n*-alkanols mediated via the lipids would be minimal.

The lack of effects of alkanols on the basal activity of adenylate cyclase as a function of temperature supports this conclusion. Alternatively, our data would suggest the absence of any direct activating effect on this protein. This suggestion is supported by the observation that the *n*-alkanols have no effect on the activity of ram sperm adenylate cyclase (P. Robberecht et al., *unpublished results*) which consists only of the catalytic subunit of the enzyme (Stengel & Hanouňe, 1981). The effects of ethanol have been extensively studied recently (Hoffman & Tabakoff, 1982; Rabin & Molinoff, 1983; Luthin & Tabakoff, 1984). Tabakoff and coworkers observed no effect of ethanol on basal activity and suggest that the nucleotide binding protein is involved in the activation of adenylate cyclase in mouse striatal membranes. In the same membrane, Rabin and Molinoff (1983) suggest multiple sites of action of ethanol.

Adenylate cyclase activity stimulated via the guanyl nucleotide regulatory protein by GTP, GppNHp or NaF is further increased by most of the alkanols tested. The increase in V_{max} occurs with no change in K_{act} as verified for GppNHp and NaF (Fig. 3*A* and *B*). One hypothesis explains this activation of adenylate cyclase through increased fluidity of the lipid matrix. This hypothesis is based a) on the relationship observed for *n*-alkanols between anesthetic potency and lipid solubility (Seeman, 1972), the latter being related to various effects on lipid dynamics (Seeman, 1972; Chin & Goldstein, 1977; Paterson et al., 1972; Lee, 1976; Jain & Wu, 1977) and b) on the demonstrated regulation of adenylate cyclase activity by the lipid matrix (Orly & Schramm 1975; Rimon et al., 1978; Gordon et al., 1980). However, our results do not support this hypothesis for rat cardiac adenylate cyclase. *Firstly,* the Arrhenius representation of the rat cardiac adenylate cyclase activity which represents at least in part the lipid-protein interactions has several characteristics (Fig. 5, Chatelain et al., 1982*b*): a break-point at 31°C with an E_a at high temperature lower than below the break in the presence of GTP activities, a straight line with an increased E_a for the GppNHp- and NaF-stimulated enzyme which are not modified by the presence of alkanols at their maximally stimulatory concentrations. The same data were obtained by Hoffman and Tabakoff (1982) with ethanol on mouse striatal adenylate cyclase. One can argue that the effect on the lipid dynamics is not sufficient to alter the enzyme activity. Data from the literature indicate, however, that in the same range of aqueous concentrations, the effect of alkanols is to shift to low temperature the phase transition temperature of dipalmitoylphosphatidylcholine by 2 to 5°C (Jain & Wu, 1977) or that, in brain membranes, reductions in order parameter occurs at nerve-blocking concentrations of alkanols (Lyon et al., 1981) suggesting that these effects are sufficient to modify the enzyme activity as shown for another adenylate cyclase system (Gordon et al., 1980). *Secondly,* according to the concept developed by Meyer and Overton, anesthesia occurs when a compound located within the hydrophobic region of a cell reaches a critical molar concentration (Seeman, 1972). Mullins (1954) proposed a modification of this rule considering the perturbations as due to molecular volume instead of to molar concentration. It follows that equivalent values in molar concentration or molecular volume should provide an equipotent effect. This is not the case when considering the effect of *n*-alkanols on rat cardiac adenylate cyclase. Indeed the higher alkanols tested (octanol, decanol) do not activate the enzyme. But, more importantly, both the alkanol membranous molar concentration and molecular volume are not constant (Table 2, Fig. 7) in their power to increase enzyme activity by 20%. Furthermore, although no relation can be established for the molar concentration, a relationship between log *P* and log molecular volume appears for the first five alkanols. This suggests that the partition coefficient is not the sole factor governing the effect of alkanols but that the volume of the molecule (i.e., the length of the hydrocarbon chain, the hydrophobic/hydrophilic balance, the distance between hydrophilic and hydrophobic centers) is of critical importance. Within a homologous series of alkanols, the first five form a group clearly distinct from the longer alkanols with respect to their effects both on the lipid dynamics (Jain & Wu, 1977) and on the ability to stimulate enzyme activities associated with the membrane-like phospholipase A_2 and galactoside transport (Jain et al., 1978). Concerning the lipids, the alkanols with one to five methylene groups in-

teract with the interior of the hydrocarbon phase of the bilayer slightly towards the polar region (Jain & Wu, 1977). *Thirdly,* the activation by alkanols of the permanently activated adenylate cyclase is difficult to reconcile with an effect on the lipid dynamics. In this case, the guanyl nucleotide regulatory protein and the catalytic unit are, in fact, permanently coupled so that phenomena capable of controlling the adenylate cyclase activity, like protein lateral diffusion (Rimon et al., 1978) or modification of acyl chain ordering (Sinensky et al., 1979) are not likely to occur.

The efficacy of secretin to stimulate rat cardiac adenylate cyclase is selectively increased by *n*-alkanols in the same range of aqueous concentations as that required for increasing the guanyl nucleotide response. No significant activation was observed for the isoproterenol- and glucagon-stimulated enzyme. Conceivably, the effects of alkanols could occur at three levels at least, namely:

a) in solution, an alkanol-secretin interaction could modify the solvation of the hormone which might change its conformation and favor recognition of the binding site. Stabilizing effect of alkanols increasing with chain-length from methanol to butanol has been demonstrated on protein (Asakura et al., 1978);

b) at the level of the receptor, the presence of alkanol may also modify its conformation by a general "nonspecific" interaction with the polypeptide but also may change the solvation of the binding site. Replacing hydration water by neutral organic solvent strongly affects the catalytic properties of some enzymes (Martinek et al., 1982; Zhang, 1983);

c) owing to the presence of alkanol within the membrane and as discussed for the guanyl nucleotide regulatory protein, the receptor-guanyl nucleotide regulatory protein might also be modified in such a way to stabilize their interaction. The β-adrenergic, glucagon and secretin receptors are different proteins (Chatelain et al., 1980). Consequently, the effects of alkanols on the conformation of the polypeptide chain and on the coupling between a given receptor and the guanyl nucleotide regulatory protein are likely to vary from one receptor to the other.

In summary, our data do not suggest an increase in rat cardiac adenylate cyclase mediated by a nonspecific effect on the lipid dynamics. Owing to the precise molecular constraint observed, it is more likely that the activating effect of alkanol could be due to a direct interaction with the proteins particularly the guanyl nucleotide regulatory protein. In view of the localization of alkanols in the bilayer, it is possible that the molecules may interfere with the polypeptide at the interface between the lipid hydrocarbon chain and the polypeptide chain. One privileged site of interaction could be in the contact region between the guanyl nucleotide regulatory protein and the catalytic unit in relation with the observed inhibition by the same alkanols of the forskolin-activated adenylate cyclase (Robberecht et al., 1983).

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