The influence of temperature and membrane-fluidity changes on the olfactory adenylate cyclase of the rat

Stephen G. SHIRLEY, C. Jane ROBINSON and George H. DODD

Olfaction Research Group, Department of Chemistry, University of Warwick, Coventry CV4 7AL, U.K.

At physiological temperatures, the activity of the olfactory adenylate cyclase of the rat is fairly insensitive to small changes in temperature. Membrane fluidization by benzyl alcohol also produces rather small modulations of cyclase activity, although this chemical also has a more specific effect on the enzyme. Insensitivity to temperature and membrane-fluidity changes are desirable properties for a transduction system which must function in an exposed environment.

INTRODUCTION

The adenylate cyclase of the olfactory mucosa is stimulated by odorants and has been implicated in olfactory transduction (Pace *et al.*, 1985; Shirley *et al.*, 1986; Sklar *et al.*, 1986). The transduction process involves a G-protein (Pace *et al.*, 1985; Shirley *et al.*, 1986), and large quantities of G_β have been found in the olfactory cilia (Anholt *et al.*, 1987). A G-protein is also found in extracts of material from this source (Pace & Lancet, 1986).

Although the nasal passages warm incoming air, the olfactory membranes would still be expected to experience temperature fluctuations *in vivo*. It is also possible that some odorants might affect the fluidity of the membranes. In many systems, the activity of adenylate cyclase and its coupling to the receptor are highly sensitive to temperature and to membrane fluidity (e.g. Engelhard *et al.*, 1976; Houslay *et al.*, 1976, 1980; Dipple & Houslay, 1978; Brasitus & Schachter, 1980; Gordon *et al.*, 1980a,b; Houslay & Gordon, 1983). If this were the case in the olfactory system, temperature and fluidity fluctuations could seriously interfere with olfactory transduction.

We have investigated the effect of temperature upon the olfactory enzyme and its activation. The effect of membrane fluidity is more difficult to study, since many of the agents used to fluidize membranes are themselves odorants, and it would be difficult to distinguish effects mediated by the olfactory receptors from those mediated by the membrane. Accordingly we have limited our study using benzyl alcohol to the maximal (GTP γ S-stimulated) activity of the enzyme. This activity should be unaffected by events occurring at the receptors. Direct measurement of membrane fluidity seemed inappropriate, because of the current crudity of the preparation.

MATERIALS AND METHODS

[α - 32 P]ATP and cyclic [8- 3 H]AMP were obtained from Amersham International (ethanol was removed, under N_2 , from the labelled ATP before use). Biochemicals were obtained from Sigma. All other reagents were of analytical quality. Male Wistar rats of about 200 g body weight were fed *ad libitum*; the fatty acid composition of the diet is not known.

Membranes were prepared as previously described (Shirley *et al.*, 1986), except that the time of sonication was shortened to 4 s and the 20000 g pellet was resuspended in phosphate-buffered saline, pH 7.5, and re-centrifuged before use. The material was stored on ice and assayed on the day of preparation. Adenylate cyclase activity was assayed in an 80 μ l final volume containing 50 mM-phosphate buffer, pH 7.5, 1 mM-ATP, about 250 nCi of [α - 32 P]ATP, 2.3 mM-MgCl $_2$, 120 μ M-EGTA, 3 mM-cyclic AMP, 1.4 mM-isobutylmethylxanthine, 40 μ M-GTP (or GTP γ S), bovine serum albumin (6.24 mg/ml), creatine phosphate (6.24 mg/ml) and creatine phosphokinase (0.625 mg/ml). The reaction was terminated by the addition of 150 μ l of HClO $_4$, and 30 nCi of cyclic [3 H]AMP were added. The radioactivity product was separated and its radioactivity counted by using the two-column method of White & Karr (1978). Since phosphate buffer has a small temperature coefficient, separate buffers were not prepared for each temperature. Instead, corrections were made to the measured rate by using the measured pH profile of the cyclase reaction. In no case did these corrections exceed $\pm 4\%$ of the measured rate. When GTP γ S or F $^-$ and Al $^{3+}$ were used, these compounds (at their final concentrations) were added to the membranes at least 2 $\frac{1}{2}$ h before the start of the assay. Assay incubation times ranged from 5 to 300 min depending on temperature, and incubations were performed at final protein concentrations of about 20 μ g/ml. Protein was determined by the method of Hartree (1972), with bovine serum albumin as standard.

RESULTS

The production of cyclic AMP was linear with time over the periods of time used for the assays and under all conditions of temperature and additions. The pre-incubation mentioned in the Materials and methods section was necessary because, immediately after the addition of F $^-$, accelerating rates were observed for a period of about 40 min. As in other systems (Sternweis & Gilman, 1982), Al $^{3+}$ potentiated the ability of F $^-$ to stimulate the cyclase (results not shown).

Fig. 1(a) shows Arrhenius plots of adenylate cyclase activity assayed in the presence of 40 μ M-GTP. The slope

Abbreviation used: GTP γ S, guanosine 5'-[γ -thio]triphosphate; phosphate-buffered saline, 154 mM-NaCl/5 mM-sodium phosphate, pH 7.5.

Fig. 1. Arrhenius plots of olfactory adenylate cyclase

(a) Shows the combined data from eight independent preparations of olfactory adenylate cyclase. The data for each preparation have been shifted vertically to superimpose the curves. In no case did this adjustment to specific activity exceed 30%. The specific-activity axis refers to the geometric mean of the activity. Each data point is the mean of duplicate or triplicate determinations, and variability between replicates was 1.9% (S.D.). The assays were performed in the presence of 40 μ M-GTP, and, although no odorant was added, we cannot exclude the possibility of an endogenous odorant or one of the assay components acting as an odorant. The curve should be regarded as an approximation to the physiological resting activity rather than to the basal activity. (b) Shows the activation of the cyclase activity (total activity in the presence of stimulus/resting activity). Reproducibility of replicate determinations was as in (a). (i) 1 mM-Acetophenone was added. The data are taken from three preparations, and no adjustment of activation is necessary to superimpose the curves. (ii) 5 mM-NaF and 20 μ M-AlCl₃ were substituted for GTP. The data are taken from one preparation and are plotted 0.5 logarithmic (ln) unit below the true value for separation from curve (iii). (iii) 40 μ M-GTP_γS was substituted for GTP. The data are taken from two preparations, and no adjustment to the activation is necessary to superimpose the curves.

Fig. 2. Effect of benzyl alcohol on olfactory adenylate cyclase

In the inset (a) the open circles show the activity of olfactory adenylate cyclase measured in the presence of $40 \mu\text{M-GTP}\gamma\text{S}$. The filled circles show the activity on the addition of $50 \text{ mM-benzyl alcohol}$. (b) shows the same data, except that the benzyl alcohol points are plotted at a temperature 4.09°C higher and a specific activity 0.580 ln unit higher than actually measured.

of the curve for each preparation at physiological temperature was determined by using the points lying between 34 and 39°C . The mean slope corresponds to an activation enthalpy of $24 (\pm 9; 95\% \text{ confidence interval}) \text{ kJ}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$. This corresponds to a temperature coefficient of $3 (\pm 1.2)\% / ^\circ\text{C}$ for the reaction or a Q_{10} between 1.2 and 1.5 . Viewing the curve as a series of straight-line segments, least-squares analysis reveals breaks of slope at 33.0 , 19.7 and 7.3°C . The slopes of the segments are 24 , 60 , 80 and $117 \text{ kJ}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$ (from high to low temperature).

Fig. 1(b) shows Arrhenius plots of the activation produced by $1 \text{ mM-acetophenone}$ and by substitution of $\text{GTP}\gamma\text{S}$ or F^- and Al^{3+} for GTP . The slopes are expressed as enthalpies to allow easy addition to the enthalpies of the resting state, but Fig. 1(b) shows activations and not absolute reaction rates. The acetophenone curve shows a break at $23.9 (\pm 1.3; 95\% \text{ confidence interval})^\circ\text{C}$, with a slope at low temperatures of $21 (\pm 2; 95\% \text{ confidence interval}) \text{ kJ}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$. Above the break, the curve is virtually flat [slope $-6.6 \pm 5.8 (95\% \text{ confidence interval}) \text{ kJ}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$]. The curve with $\text{GTP}\gamma\text{S}$ is of the same shape, with a break at $17.4 (\pm 3.0)^\circ\text{C}$ and slopes of $43 (\pm 7)$ and $8 (\pm 7) \text{ kJ}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$. The curve with F^- and Al^{3+} is of the same general shape, but straight-line segments are a poorer approximation.

Benzyl alcohol inhibited the $\text{GTP}\gamma\text{S}$ -stimulated acti-

Fig. 3. Variation of adenylate cyclase activity with benzyl alcohol concentration

Each point is the mean of duplicate determinations whose s.d. was 1.9% . The adenylate cyclase activity was measured in the presence of $40 \mu\text{M-GTP}\gamma\text{S}$. The 100% points correspond to rates of 2.65 pmol/min (10°C), 21.2 pmol/min (25°C) and 52.3 pmol/min (38°C).

vity at high temperatures and stimulated it at low ones (Figs. 2 and 3). However, the Arrhenius plots of activity with and without benzyl alcohol could be superimposed by assuming that the effect of the reagent was to shift the curve by 4.09°C and to inhibit the reaction by 46% (Fig. 2).

DISCUSSION

If the olfactory cyclase *in vivo* shows the same behaviour as we have found *in vitro*, then the very small values of Arrhenius slopes at physiological temperatures would make olfactory transduction almost temperature-independent. In the presence of acetophenone, the total cyclase activity shows an activation enthalpy of only $17 \text{ kJ}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$. *In vivo*, the olfactory system may not be called upon to discriminate odour from no odour but only odour from background, in which case this even lower value for activation enthalpy would be the relevant one.

The addition of benzyl alcohol at 40 mM to membranes is usually equivalent to a temperature increase of $6\text{--}8^\circ\text{C}$ (Dipple & Houslay, 1978; Brasitus & Schachter, 1980; Gordon *et al.*, 1980a). In the case of the olfactory preparation, $50 \text{ mM-benzyl alcohol}$ produced an effect equivalent to only 4.1°C . This small shift, coupled with the low Arrhenius slopes, would make the olfactory cyclase fairly insensitive to modulation via membrane fluidity. For example, $50 \text{ mM-benzyl alcohol}$ (acting as a membrane fluidizer), causing an effect equivalent to 4.1°C coupled with a temperature coefficient of $3\% / ^\circ\text{C}$ would stimulate the cyclase by 12% , which is the stimulation produced by $5 \mu\text{M-acetophenone}$ (acting as

an odorant) (Shirley *et al.*, 1986). Furthermore, temperature or fluidity changes would be expected to affect all of the cyclase activity equally, whereas an odorant, acting via a limited subset of receptors, would stimulate only part of it and would be recognized by the pattern of differential stimulation.

Inhibition of adenylate cyclase by benzyl alcohol (at higher concentrations) has been observed in rat liver (Gordon *et al.*, 1980a,b) and has been attributed to the alcohol competing for protein domains normally occupied by annular lipid (Gordon *et al.*, 1980a). Freeze-etch studies of the olfactory cilia revealed a large number of particles in the membrane (Menco, 1976, 1977, 1980), implying that these membranes are protein-rich. An unusually high ratio of annular to non-annular lipid might explain why the inhibitory effect of benzyl alcohol is more prominent with the olfactory cyclase. Also, the inhibitory effect of benzyl alcohol may be more visible in the olfactory case, since the membrane-fluidization (stimulatory) effect is smaller.

It would be premature to try to assign meanings to breaks of slope in the Arrhenius plot of resting activities, if any indeed exist; the graph can equally well be fitted by a smooth curve. However, the curve seems to lack the break of slope at about 25 °C that has been found in many cyclase systems (Bar, 1974; Orly & Schramm, 1975; Engelhard *et al.*, 1976; Pliego & Rubalcava, 1978; Rene *et al.*, 1978; Brasitus & Schachter, 1980; Houslay, 1978). This change of slope seems characteristic of the cyclase catalytic unit (Houslay & Gordon, 1983), and its absence might suggest that the olfactory enzyme may be slightly unusual form. But there is another possible explanation. In other systems, this break of slope is abolished by stimulation with relevant ligands and guanine nucleotides (Bar, 1974; Engelhard *et al.*, 1976; Pliego & Rubalcava, 1978; Rene *et al.*, 1978; Brasitus & Schachter, 1980; Houslay, 1981). The olfactory cyclase (measured without the addition of odorants but with GTP) may, in fact, be stimulated, either by endogenous odorants or by a component of the assay system.

Acetophenone at 1 mM is not a saturating concentration of odorant. Indeed, saturation has not been demonstrated in olfaction by biochemical means (Pace *et al.*, 1985; Shirley *et al.*, 1986) or by electrophysiology. The receptor sites are, therefore, not fully occupied by odorant, and the degree of occupancy may well change with temperature. A high degree of receptor heterogeneity is also suspected in the olfactory system (Polak, 1973; Pace *et al.*, 1985; Shirley *et al.*, 1986). Accordingly,

the break of slope in the acetophenone activation curve, although well-defined, should, at the moment, only be taken as characteristic of this odorant at this concentration.

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