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The local anaesthetic benzyl alcohol attenuates the α_2-adrenoceptor-mediated inhibition of human platelet adenylate cyclase activity when stimulated by prostaglandin E_1, but not that stimulated by forskolin

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Treatment of human platelets with concentrations of benzyl alcohol up to 50 mM augmented adenylate cyclase activity when it was assayed in the basal state and when stimulated by prostaglandin E_1 (PGE_1), isoprenaline or NaF. Benzyl alcohol antagonized the stimulatory effect exerted on the catalytic unit of adenylate cyclase by the diterpene forskolin. Benzyl alcohol did not modify the magnitude of the inhibitory response when the catalytic unit of adenylate cyclase was inhibited by using either low concentrations of guanosine 5'-[$\beta\gamma$-imido]triphosphate, which acts selectively on the inhibitory guanine nucleotide-regulatory protein G_i, or during α_2-adrenoceptor occupancy, by using adrenaline (+propranolol). Some 34% of the potent inhibitory action of adrenaline on PGE_1-stimulated adenylate cyclase was obliterated in a dose-dependent fashion (concn. giving 50% inhibition = 12.5 mM) by benzyl alcohol, with the residual inhibitory action being apparently resistant to the action of benzyl alcohol at concentrations up to 50 mM. Treatment of membranes with benzyl alcohol did not lead to the release of either the α-subunit of G_i or G-protein β subunits. The α_2-adrenoceptor-mediated inhibition of adenylate cyclase was abolished when assays were performed in the presence of Mn^{2+} rather than Mg^{2+} and, under such conditions, dose–effect curves for the action of benzyl alcohol on PGE_1-stimulated adenylate cyclase activity were similar whether or not adrenaline (+propranolol) was present. We suggest that (i) α_2-adrenoceptor- and G_i-mediated inhibition of PGE_1-stimulated adenylate cyclase may have two components, one of which is sensitive to inhibition by benzyl alcohol, and (ii) the G_i-mediated inhibition of forskolin-stimulated adenylate cyclase exhibits predominantly the benzyl alcohol-insensitive component.

INTRODUCTION

Adenylate cyclase is a multicomponent enzyme system which is responsible for mediating the intracellular actions of a wide variety of hormones [1–4]. In human platelets this enzyme is under dual control, where distinct receptors can exert either stimulatory effects, mediated by the guanine nucleotide-regulatory protein G_s, or inhibitory effects, mediated by a distinct G-protein termed G_i [1,5–9]. These G-proteins are heterotrimeric species consisting of an α (GTP-binding) subunit, together with β and γ components which dissociate upon activation. For G_s and the various forms of G_i, it appears that the β and γ subunits are identical [1–3].

Receptors appear able to exert inhibitory effects on adenylate cyclase through G_i in two distinct ways [1–3]. Firstly inhibition is believed to occur as a result of a direct effect of α-G_i (α subunit of G_i) on the catalytic subunit of adenylate cyclase. Secondly, the β–γ subunits released from G_i will serve to attenuate, by mass action, the dissociation of G_s. However, the relative magnitudes of the inhibitory effects exerted by these two routes in particular systems are unclear.

In this study we use human platelets to determine the effect of the neutral local anaesthetic benzyl alcohol on the receptor-mediated inhibitory responses exerted on adenylate cyclase. We show that benzyl alcohol exerts very different effects on the G_i-mediated inhibition of this enzyme, which depends on whether adenylate cyclase is being stimulated by G_s or not.

MATERIALS AND METHODS

PGE_1, isoprenaline, adrenaline, propranolol and bovine serum albumin were obtained from Sigma Chemical Co., Poole, Dorset, U.K. Forskolin was from Calbiochem, and radiochemicals were purchased from Amersham International, Amersham, Bucks., U.K. All other biochemicals were from Boehringer (U.K.) Ltd., Lewes, East Sussex, U.K., with all other chemicals being of A.R. grade from BDH Chemicals, Poole, Dorset, U.K.

Blood was obtained from the antecubital vein of normal healthy volunteers who were not undergoing medication (BTS, St. Vincent Street). Platelets were prepared as in ref. [10] and membranes were prepared as described in ref. [11]. A solubilized protein extract from platelet membranes was obtained by using the detergent Lubrol as described in detail previously by us [12].

Adenylate cyclase assays were carried out as previously [12–14], being performed at final pH 7.4 in

Abbreviations used: PG, prostaglandin; EC_{50}, concentration of ligand giving 50% activation; IC_{50}, concentration of ligand giving 50% inhibition; p[NH]ppG, guanosine 5'-[$\beta\gamma$-imido]triphosphate.

Table 1. Activity of adenylate cyclase in human platelets

Assays were done in the presence of forskolin (0.1 mM), NaF (15 mM), PGE_1 (10 μM) and isoprenaline (0.1 mM). Specific activity is given in pmol/min per mg of protein. Fold stimulation is the fold increase in activity over that seen in the absence of any added ligand (basal). Abbreviations: C, catalytic unit of adenylate cyclase; G_s, stimulatory guanine nucleotide-regulatory protein. Results are means ± S.D. for the numbers of different membrane preparations shown.

Ligand	Components involved	No. of observations	Sp. activity	Stimulation (fold)
None (basal)	C	6	8.7 ± 1.3	(-)
Forskolin	C	6	270.4 ± 41.1	31.0
NaF	G_s–C	4	71.6 ± 7.9	8.2
PGE_1	PGE_1 receptor–G_s–C	14	234.5 ± 35.5	26.7
Isoprenaline	β-adrenoceptor	5	14.1 ± 4.1	1.6

a 100 μl volume containing final concentrations of 25 mM-triethanolamine/HCl, 1 mM-EDTA, 10 mM-theophylline, 5 mM-$MgSO_4$, 1 mM-dithiothreitol, 0.2 mg of creatine kinase/ml, 1 mM-ATP, 0.8 mg of albumin/ml and 7.2 mg of phosphocreatine/ml. The cyclic AMP produced was measured as described previously by using the cyclic-AMP-binding subunit of protein kinase prepared from bovine heart in a binding assay. In all assays initial rates from linear time courses were determined under all conditions. Protein was determined in accordance with ref. [15], as modified in ref. [16], with bovine serum albumin as a standard.

In order to determine whether G-protein components were released during exposure to benzyl alcohol in the assay, they were incubated in adenylate cyclase assay buffer (with or without 50 mM-benzyl alcohol), and 25 μg of soya-bean trypsin inhibitor was added as a carrier protein before they were centrifuged at 126000 *g* for 2 min on a TL-100 Beckman ultracentrifuge. Membrane pellets were solubilized in SDS sample buffer as in ref. [17]. Protein was precipitated from the supernatant fraction by using deoxycholate/trichloroacetic acid precipitation before being solubilized in SDS sample buffer [17]. After SDS/polyacrylamide-gel electrophoresis [10% (w/v) acrylamide], under reducing conditions, proteins were transferred to nitrocellulose and immunoblotting was performed. These procedures have been used to assess release of G-protein subunits from membranes previously and have been detailed elsewhere [17]. Immunoblotting was then performed with an antiserum RV6 directed against the G-protein β-subunits found associated with a mixture of G_i and G_o, and also with the anti-peptide antiserum AS7, directed against the *C*-terminal decapeptide of transducin, to detect α-G_i [17,18].

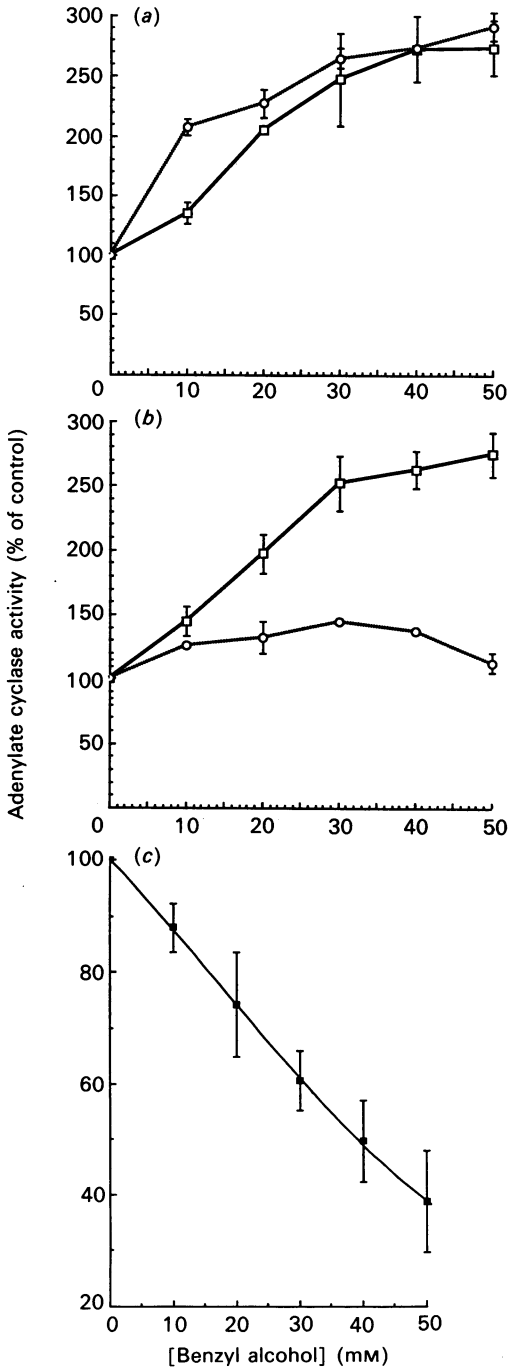

Fig. 1. Action of benzyl alcohol on basal and ligand-stimulated adenylate cyclase activity

(*a*) Basal activity (○) and that stimulated by isoprenaline (0.1 nM) (□); (*b*) stimulated by NaF (15 mM) (□) and (○) stimulated by PGE_1 (20 μM); (*c*) stimulated by forskolin (0.1 mM). Each 100 μl of assay contained 2 mg of platelet membrane protein. Results are means ± S.D. for $n = 6$ separate experiments using different platelet membrane preparations. Specific activities are given in Table 1. Activity in the absence of added benzyl alcohol (control) is shown as 100%.

Table 2. Stimulatory action of benzyl alcohol on platelet adenylate cyclase activity

Maximum stimulation of adenylate cyclase activity is given compared with activity monitored in the absence of this agent (control = 100%). The ligand-stimulated increase in adenylate cyclase activity is given as a fold increase over that of the basal for experiments done either in the absence of benzyl alcohol or in the presence of maximally stimulating concentrations of this ligand. Results are means ± S.D. for $n = 6$ experiments using different membrane preparations. Specific activities are given in Table 1.

Regulatory ligand	Maximal stimulation by benzyl alcohol (%)	Ligand-mediated stimulation (fold increase over basal)	
		Absence of benzyl alcohol	Presence of benzyl alcohol
None (basal)	311 ± 7	(1)	(1)
NaF (15 mM)	262 ± 22	8.2	6.9
PGE_1 (20 μM)	144 ± 5	26.7	12.5
Isoprenaline (0.1 mM)	294 ± 6	1.6	1.5

RESULTS

The specific activities of adenylate cyclase in human platelet membranes are given in Table 1 for the basal and various ligand-stimulated states. These include the diterpene forskolin, which is believed to exert its effect directly upon the catalytic unit of adenylate cyclase [19], NaF, which activates G_s directly [1–3], and the ligands PGE_1 and isoprenaline, which exert their effects through specific stimulatory receptors coupled to G_s [1,3]. The ability of these various ligands to stimulate adenylate cyclase activity was markedly different, with forskolin and PGE_1 being the most potent activators (Table 1).

Concentrations of up to 50 mM-benzyl alcohol were employed, at which point benzyl alcohol has been shown to partition into biological membranes and elicit an increase in bilayer fluidity which is equivalent to a temperature rise of approx. 6–8 °C, as determined in various model and biological membranes, including human platelets [20–23]. Increasing concentrations of benzyl alcohol enhanced basal adenylate cyclase activity as well as that observed in the presence of NaF (15 mM), isoprenaline (0.1 mM) or PGE_1 (20 μM) (Fig. 1). Benzyl alcohol had little effect on the fold stimulation (stimulated/basal activity ratio) of adenylate cyclase activity achieved by either isoprenaline or NaF (Table 2). However, the presence of benzyl alcohol decreased the fold stimulation of adenylate cyclase activity by PGE_1 (Table 2). When a Lubrol-solubilized extract of platelet membranes was used, benzyl alcohol, at concentrations up to 50 mM, failed to affect (less than 5% difference) basal adenylate cyclase activity. In contrast, when the diterpene forskolin was used to stimulate the catalytic unit of adenylate cyclase, then increasing concentrations of benzyl alcohol led to a progressive inhibition of adenylate cyclase activity (Fig. 1). Benzyl alcohol exerted little effect on the EC_{50} value for activation of adenylate cyclase by forskolin, which was 21 ± 13 μM in the absence of benzyl alcohol and 23 ± 10 μM in the presence of benzyl alcohol ($n = 3$ experiments with different membrane preparations; means ± S.D.). It did, however, increase the EC_{50} for activation of adenylate cyclase by PGE_1 which, in the absence of benzyl alcohol, was 0.28 ± 0.09 μM-PGE_1 (mean ± S.D. for $n = 3$ separate experiments with different membrane preparations).

G_i-mediated effects on adenylate cyclase were examined under conditions where this inhibitory G-protein was activated either directly, by using low concentrations of the inhibitory GTP analogue p[NH]ppG [24], or indirectly, by virtue of occupancy of the α_2-adrenergic inhibitory receptor. Thus, in one instance, adenylate cyclase was first stimulated with PGE_1 (20 μM) and inhibition was then achieved through the α_2-adrenoceptor route by using adrenaline (100 μM) in the presence of the β-adrenoceptor antagonist propranolol (10 μM). This achieved a 79.5 ± 1.1% inhibition of activity ($n = 5$; mean ± S.D.). Alternatively, forskolin was used to stimulate the catalytic unit of adenylate cyclase, and inhibition was elicited by using either adrenaline together with propranolol (50.0 ± 3.5% inhibition) or low concentrations of p[NH]ppG (10 nM), which selectively activate [24] the inhibitory G-protein, G_i (53.5 ± 5.0% inhibition).

When either p[NH]ppG or adrenaline (+propranolol) was used to inhibit forskolin-stimulated adenylate cyclase, then increasing benzyl alcohol concentrations had no discernible effect on the degree of inhibition elicited (Figs. 2*a* and 2*b*). Indeed, the IC_{50} for inhibition of forskolin-stimulated adenylate cyclase by p[NH]ppG was 0.20 ± 0.04 μM in the absence of benzyl alcohol and 0.28 ± 0.05 μM in the presence of 50 mM-benzyl alcohol ($n = 3$; means ± S.D.). In contrast with this, however, increasing benzyl alcohol concentrations diminished the ability of adrenaline (+propranolol) to inhibit PGE_1-stimulated adenylate cyclase activity (Fig. 2*c*). The attenuating effect of benzyl alcohol exhibited an IC_{50} of 12.5 ± 4.2 mM (mean ± S.D., $n = 6$ different membrane preparations). At 50 mM-benzyl alcohol the inhibitory effect of adrenaline (+propranolol) was decreased from a 79% inhibition of activity to approx. 52% inhibition. We noted that the IC_{50} value for inhibition of adenylate cyclase by adrenaline (+propranolol) was increased from 67 ± 15 μM in the absence of benzyl alcohol to 130 ± 38 μM in its presence ($n = 4$ experiments with different membrane preparations; means ± S.D.; data are significantly different at $P < 0.01$).

In the presence of Mn^{2+}, G_i is believed to be unable to inhibit adenylate cyclase [1,25]. Here we see that, when adenylate cyclase assays were performed in the presence of Mn^{2+} (5 mM) rather than Mg^{2+} (5 mM), then PGE_1-stimulated activity was increased by some 1.8-fold (Fig. 2*d*) and the presence of adrenaline (+propranolol) in the

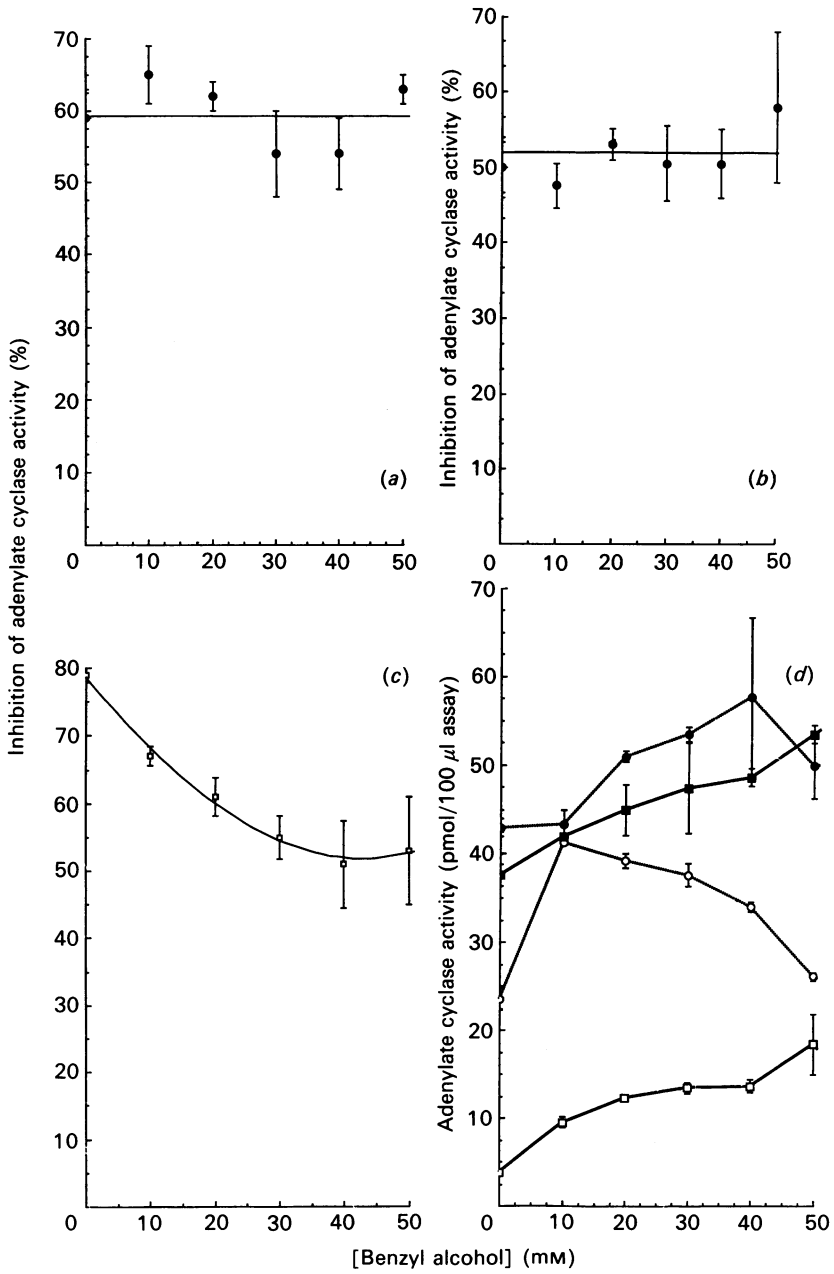

Fig. 2. Action of benzyl alcohol on ligand-mediated inhibition of adenylate cyclase activity

The effect of benzyl alcohol is shown on the percentage inhibition of forskolin (0.1 mM)-stimulated activity elicited by either (*a*) low (10 nM) concentrations of p[NH]ppG or (*b*) adrenaline (0.1 mM) + propranolol (10 μM), and (*c*) shows the effect of benzyl alcohol on the percentage inhibition of PGE_1 (20 μM)-stimulated activity elicited by adrenaline and propranolol. In (*d*) the effect of benzyl alcohol on the adenylate cyclase activity (pmol/min per 100 μl assay) profiles for PGE_1-stimulated activity in the presence of either Mg^{2+} (○, □) or Mn^{2+} (●, ■) is shown for experiments done in either the presence (□, ■) or absence (○, ●) of adrenaline (0.1 mM) + propranolol (10 μM). Results are means ± S.D. for $n = 6$ separate experiments using different membrane preparations. Specific activities are given in Table 1.

assay elicited only a 16 ± 4% inhibition of PGE_1-stimulated activity, compared with 79 ± 5% seen in its absence. Both of these observations are compatible with a loss of functional G_i. Indeed, when Mn^{2+} was used in the assays, dose–effect curves for PGE_1-stimulated activity to benzyl alcohol were very similar whether or not adrenaline (propranolol) was present (Fig. 2*d*).

If inhibition of PGE_1-stimulated adenylate cyclase activity by adrenaline + propranolol was performed at 35 °C and 40 °C, rather than 30 °C, then the magnitude of inhibition was 77 ± 9% and 82 ± 8% respectively (means ± S.D., $n = 5$).

All of the actions of benzyl alcohol were reversible upon dilution or washing the membranes to remove this ligand.

As shown by other [17], immunoblotting with the anti-peptide antiserum identified a single band indicative of α-G_i (Fig. 3). No change (less than 8%) occurred in the amount of α-G_i in platelet membrane fractions treated with benzyl alcohol (50 mM) in the presence or absence of

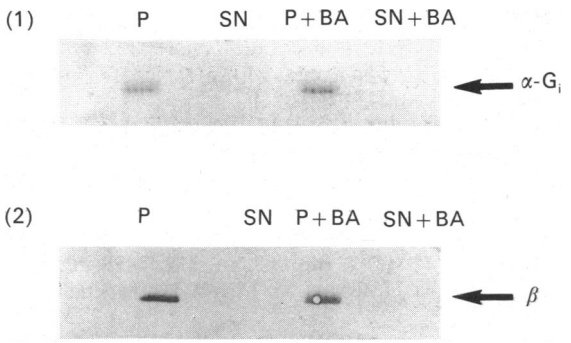

Fig. 3. Immunoblotting of platelet membranes

After incubation of platelet membranes either with or without benzyl alcohol (50 mM) under the conditions specified in the Materials and methods section, membranes and the supernatant fraction were isolated and immunoblotted for either G-protein β subunits (panel 2), with the antiserum RV6, or for α-G_i subunits (panel 1), with the antiserum AS7. The data show a typical experiment of one done three times with different membrane preparations. In the experiments shown, incubations were done in the presence of PGE_1 (20 μM), adrenaline (0.1 mM) and propranolol (10 μM). Similar results were found in the absence of PGE_1 with or without forskolin (0.1 mM) and in the absence of any of these ligands. Abbreviations: P, pellet; SN, supernatant; BA, benzyl alcohol.

PGE_1 and adrenaline (+propranolol). Similarly, we failed to detect any immunoreactive material released into the supernatant fraction of platelet membranes treated with 50 mM-benzyl alcohol (Fig. 3). Essentially identical results were found by using the antiserum to detect G-protein β-subunits (Fig. 3).

DISCUSSION

As has been shown by us [12,20–22,26,27] and others [28–30], using a variety of membrane systems, the activity of adenylate cyclase in the basal and ligand-stimulated states is enhanced in the presence of the local anaesthetic benzyl alcohol. We show here that platelet adenylate cyclase is no exception (Fig. 1). The augmentation of activity is seen when monitoring the basal catalytic activity, and also when G_s is directly stimulated with NaF and when stimulated by receptor–G_s coupling invoked by either PGE_1 or the β-adrenoceptor agonist isoprenaline. In hepatocytes, however, although basal adenylate cyclase activity was relatively insensitive to the action of benzyl alcohol, the glucagon-stimulated activity was markedly enhanced [12]. Thus benzyl alcohol profoundly amplified the ability of glucagon to stimulate the activity of adenylate cyclase (i.e. fold stimulation increased). Here, however, we observed that benzyl alcohol exerted a potent stimulatory effect on the basal activity of human platelet adenylate cyclase. This may reflect tissue and species differences in either the enzyme itself or its incorporation into the membrane bilayer. We presume that the stimulatory effect of benzyl alcohol is related to its ability to decrease membrane order [12,20–22,31], as we failed to observe any stimulatory effect of benzyl alcohol with the detergent-solubilized enzyme. Benzyl alcohol enhanced the degree of NaF-stimulation of adenylate cyclase to a similar extent to that seen for basal activity. This suggests that the amplification seen is due to an effect predominantly on the catalytic unit of the enzyme, and indicates that coupling between G_s and adenylate cyclase was not influenced by the increase in order elicited by benzyl alcohol. Indeed, despite mobile collisions between the protein components being required for this process [32], it has been shown (see [20,33]) that at the temperatures of assay (30 °C) diffusion is not a rate-limiting step in the reaction, and thus any decrease in membrane order is unlikely to elicit any significant facilitation of the rate of reaction. Thus the membrane environment does not appear to exert any constraint on the conformational flexibility of G_s, which is not entirely unexpected, as at least the α-subunit of G_s is not an integral membrane protein [1–4]. As with NaF stimulation, the presence of benzyl alcohol did not augment the net (fold) stimulation of basal adenylate cyclase activity by isoprenaline (Table 2). Thus the enhanced activity seen here may again be due predominantly to an effect on the catalytic unit of adenylate cyclase. In contrast, the fold increase in stimulation of adenylate cyclase by PGE_1 was attenuated by the presence of benzyl alcohol (Table 2). Such experiments imply that there are profound differences in the ability of benzyl alcohol to modify the efficiency of coupling of various stimulatory receptors to adenylate cyclase. This does not appear to be mediated by any change in affinity of the ligands for the receptor but, rather, in their potency to couple and activate G_s.

Benzyl alcohol (Fig. 1), like ethanol [34–36], was seen to inhibit adenylate cyclase when the enzyme was activated by the diterpene forskolin (Fig. 1). It has been suggested [34,35] that ethanol might elicit this effect by competing for the hydrophobic site on adenylate cyclase to which forskolin binds. However, we note here that benzyl alcohol did not affect the EC_{50} value for activation of adenylate cyclase by forskolin. This suggests that benzyl alcohol is acting as a non-competitive, rather than competitive, inhibitor of forskolin action, and thus interacting at a different site(s) to forskolin on the catalytic unit of human platelet adenylate cyclase.

G_i-mediated inhibitory effects on adenylate cyclase can be elicited and monitored in two distinct ways. Firstly, one can determine any direct action on the catalytic unit of adenylate cyclase by monitoring action on the forskolin-amplified basal activity [1–3,24,36]. In this instance inhibition can be engendered by selectively activating G_i with low concentrations of p[NH]ppG, because this G-protein has a much higher affinity for p[NH]ppG than does G_s [24,37]. Direct inhibitory effects on the catalytic unit can also be measured by assessing the ability of the α_2-adrenoceptors, which couple to G_i, to inhibit the forskolin-stimulated activity [6,7,9]. For both receptor- and p[NH]ppG-mediated inhibitory effects on the catalytic unit of adenylate cyclase activity we observed a complete insensitivity to benzyl alcohol at concentrations up to 50 mM. In contrast with this, the α_2-adrenoceptor-mediated inhibition of receptor-stimulated adenylate cyclase was attenuated on addition of benzyl alcohol. This occurred in a saturable fashion, with a maximal attenuation of approx. 34% being achieved. Thus the α_2-adrenoceptor inhibition of receptor–G_s-stimulated adenylate cyclase appeared to exhibit two components, one of which was insensitive to benzyl alcohol and the other which was attenuated by benzyl alcohol. The attenuating effect of benzyl alcohol was

clearly targeted at a fraction of the inhibitory G_i component. This could be shown by performing experiments in the presence of Mn^{2+}, rather than Mg^{2+}, which serves to uncouple G_i-mediated responses [1,25]. Under such conditions we observed an increase in PGE_1-stimulated activity, owing to a loss of inhibitory input, a near obliteration of the α_2-mediated inhibition of PGE_1-stimulated adenylate cyclase, and a response of PGE_1-stimulated adenylate cyclase to benzyl alcohol which was similar, whether or not adrenaline (+propranolol) was present to interact with α_2-adrenoceptors (Fig. 2).

These two inhibitory components may reflect a benzyl alcohol-insensitive component, which we suggest may reflect the direct inhibitory action of α-G_i on the catalytic unit of adenylate cyclase, and a benzyl alcohol-sensitive component, which we suggest may reflect an action on the β–γ-subunit-mediated inhibitory effect of G_i on G_s dissociation. Thus, in the absence of ligands stimulating G_s, only the benzyl alcohol-insensitive response may be observed.

We cannot define unequivocally whether this attenuating effect of benzyl alcohol was due either to enhanced bilayer fluidity or to a direct action of benzyl alcohol itself. However, the 'fluidizing' effect of 50 mM-benzyl alcohol can be mimicked by increasing the temperature by some 6–8 °C, yet such an increase in temperature did not diminish the potency of adrenaline (+propranolol) to inhibit PGE_1-stimulated adenylate cyclase activity. This suggests that benzyl alcohol attenuates the inhibitory effect of α_2-adrenoceptor occupancy on PGE_1-stimulated activity by a direct action. Such an attenuation of inhibitory action was not due to benzyl alcohol causing a loss of components of the G_i system from the membrane, as has been noted under certain conditions by others [17]. For we were able to show that the effects of benzyl alcohol were reversible on dilution or washing, and immunoblotting studies clearly indicated that both α-G_i and β subunits were not displaced by benzyl alcohol under the conditions of the assay.

The mechanism of local anaesthesia remains unknown. However, it occurs [38,39] at similar concentrations (20–35 mM) of benzyl alcohol to those required (EC_{50} 12.5 nM) to attenuate the inhibitory effect of adrenaline (+propranolol) on PGE_1-stimulated adenylate cyclase.

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REFERENCES

1. Gilman, A. G. (1987) Annu. Rev. Biochem. **56**, 615–649
2. Houslay, M. D. (1984) Trends Biochem. Sci. **9**, 39–40
3. Birnbaumer, L., Codina, J., Mattera, R., Cerione, R. A., Hildebrandt, J. D., Sunya, T., Rojas, F. J., Caron, M. J., Lefkowitz, R. J. & Iyengar, R. (1985) Mol. Aspects Cell. Regul. **4**, 131–182
4. Milligan, G. (1988) Biochem. J. **255**, 1–13
5. Houslay, M. D., Bojanic, D. & Wilson, A. (1986) Biochem. J. **234**, 737–740
6. Houslay, M. D., Bojanic, D., Gawler, D., O'Hagan, S. & Wilson, A. (1986) Biochem. J. **238**, 109–113
7. Jakobs, K. H., Aktories, K., Minuth, M. & Schultz, G. (1985) Adv. Cyclic Nucleotide Res. **18**, 137–150
8. Haslam, R. J., Davidson, M. M. L. & Desjardins, J. V. (1978) Biochem. J. **176**, 83–95
9. Lenox, R. H., Ellis, J., Van Riper, D. & Ehrlich, Y. H. (1985) Mol. Pharmacol. **27**, 1–9
10. MacIntyre, D. E. & Pollock, W. K. (1983) Biochem. J. **212**, 433–437
11. Jakobs, K. H., Lasch, P., Minuth, M., Aktories, K. & Schultz, G. (1982) J. Biol. Chem. **257**, 2829–2833
12. Dipple, I. & Houslay, M. D. (1978) Biochem. J. **174**, 179–190
13. Houslay, M. D., Metcalfe, J. C., Warren, G. B., Hesketh, T. R. & Smith, G. A. (1976) Biochim. Biophys. Acta **436**, 489–494
14. Heyworth, C. M. & Houslay, M. D. (1983) Biochem. J. **214**, 93–98
15. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) J. Biol. Chem. **193**, 265–275
16. Petersen, G. L. (1977) Anal. Biochem. **83**, 346–356
17. Milligan, G., Mullaney, I., Unson, C. G., Marshall, L., Spiegel, A. M. & McArdle, H. (1988) Biochem. J. **254**, 391–396
18. Milligan, G., Davies, S. A., Houslay, M. D. & Wakelam, M. J. O. (1989) Oncogene **4**, 659–663
19. Seamon, K. B. & Daly, J. W. (1982) J. Biol. Chem. **257**, 11591–11596
20. Houslay, M. D. & Gordon, L. M. (1983) Curr. Top. Membr. Transp. **18**, 179–231
21. Houslay, M. D. (1985) Proc. Nutr. Soc. **44**, 157–165
22. Gordon, L. M., Sauerheber, R. D., Esgate, J. A., Dipple, I., Marchmont, R. J. & Houslay, M. D. (1980) J. Biol. Chem. **255**, 4519–4527
23. Colley, C. M. & Metcalfe, J. C. (1972) FEBS Lett. **24**, 241–246
24. Hildebrandt, J. D., Hanoune, J. & Birnbaumer, L. (1982) J. Biol. Chem. **257**, 14723–14725
25. Hoffman, B. B., Yim, S., Tsai, B. S. & Lefkowitz, R. J. (1981) Biochem. Biophys. Res. Commun. **100**, 724–731
26. Gordon, L. M., Whetton, A. D., Rawal, S., Esgate, J. A. & Houslay, M. D. (1983) Biochim. Biophys. Acta **729**, 104–114
27. Needham, L. & Houslay, M. D. (1982) Biochem. J. **206**, 89–95
28. Brasitas, T. A. & Schachter, D. (1980) Biochemistry **19**, 2763–2769
29. Friedlander, G., Le Grimellec, C., Giocondi, M. C. & Amiel, C. (1987) Biochim. Biophys. Acta **903**, 345–348
30. De Foresta, B., Rogard, M., Le Maire, M. & Gallay, J. (1987) Biochim. Biophys. Acta **905**, 240–256
31. Gordon, L. M., Mobley, P. W., Esgate, J. A., Hofmann, G., Whetton, A. D. & Houslay, M. D. (1983) J. Membr. Biol. **76**, 139–149
32. Houslay, M. D., Dipple, I. & Elliott, K. R. F. (1980) Biochem. J. **186**, 649–658
33. Henis, Y. I., Rimon, G. & Felder, S. (1982) J. Biol. Chem. **257**, 1407–1411
34. Whetton, A. D., Needham, L., Dodd, N. J. F., Heyworth, C. M. & Houslay, M. D. (1983) Biochem. Pharmacol. **32**, 1601–1608
35. Robberecht, P., Waelbroeck, M., Chatelain, P., Camus, J. C. & Christophe, J. (1983) FEBS Lett. **154**, 205–208
36. Huang, R. D., Smith, M. F. & Zahler, W. L. (1982) J. Cyclic Nucleotide Res. **8**, 385–394
37. Gawler, D., Milligan, G., Spiegel, A. M., Unson, C. G. & Houslay, M. D. (1987) Nature (London) **327**, 229–232
38. Sieman, P. (1972) Pharmacol. Rev. **24**, 583–655
39. Schauf, C. & Agin, D. (1969) Nature (London) **221**, 768–769

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