The detergent Solulan C-24 reveals properties of the olfactory adenylate cyclase system

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The detergent Solulan C-24 has been shown to activate the olfactory adenylate cyclase, with loss of the odorant modulation, at concentrations too low to cause significant solubilization. The activation is synergistic with that of nonhydrolysable GTP analogues, forskolin and AlF_4^- . These effects are not reversible. Solulan causes the cyclase activity to become subject to ATP inhibition, which is competitively relieved by $\text{GTP}\gamma\text{S}$, and increases the $\text{GTP}\gamma\text{S}$ concentration required for half-maximal stimulation of the system. This suggests a change in the GTP-binding site of the stimulatory G-protein. Activation by $\text{GTP}\gamma\text{S}$, without Solulan, indicates that the cyclase catalytic unit, rather than the available G-protein, may be limiting in the system. We suggest that Solulan may remove an inhibitory control on the cyclase activity.

INTRODUCTION

In the study of adenylate cyclase systems, detergents are often used in the extraction and purification of the components. The detergents can have a significant effect on the activities and properties of the proteins. Sutherland *et al.* (1962) found, for example, that Triton inhibits the activity of the adenylate cyclase derived from several cell types, whilst others have reported activation by non-ionic detergents [Johnson & Sutherland, 1973; McDonald, 1975; Perkins & Moore, 1971; for a review, see Neer (1978)]. We have previously shown that the activity of the olfactory adenylate cyclase of the rat can be enhanced by non-ionic detergents (Shirley *et al.*, 1987), and we now report the use of the detergent Solulan C-24, together with other activators of adenylate cyclase, as a probe for studying the mechanism of control of the cyclase catalytic unit by the G-protein.

MATERIALS AND METHODS

$[\alpha\text{-}^{32}\text{P}]\text{ATP} > 15 \text{ TBq/mmol}$ and cyclic $[8\text{-}^3\text{H}]\text{AMP}$ 1.1 TBq/mmol were bought from Amersham International. Solulan C-24 [Choleth-24, poly(ethylene glycol) C-24 cholesterol ether, and Ceteth-24, poly(ethylene glycol) C-24 cetyl alcohol ether], lot no. 214, was a generous gift from Amerchol Corporation, Edison, NJ, U.S.A. Biochemicals were bought from Sigma, and all other reagents were of analytical quality.

Olfactory membranes were prepared from male Wistar rats of about 200 g body weight, as previously described (Shirley *et al.*, 1986) except that the sonication time was 3 s, and the 20000 g pellet was resuspended in phosphate-buffered saline (5 mM-sodium phosphate/154 mM-NaCl, pH 7.5) and recentrifuged before use. Membrane preparations were stored on ice and assayed on the day of preparation. All preparations were tested with acetophenone (in the presence of $40 \mu\text{M}$ -GTP) and showed normal odour-activated cyclase activity.

Adenylate cyclase activity was assayed in a final volume of $80 \mu\text{l}$ containing 50 mM-sodium phosphate buffer, pH 7.5, 1 mM-ATP, about 250 nCi of $[\alpha\text{-}^{32}\text{P}]\text{ATP}$, 3 mM- MgCl_2 , $120 \mu\text{M}$ -EGTA, 3 mM-cyclic AMP, 1 mM-isobutyl-methylxanthine, creatine phosphate (6 mg/ml) and creatine phosphokinase (0.6 mg/ml). The assay mixture also contained guanine nucleotides or other activators as detailed in the text. Reactions were performed for 15–45 min at 30°C , stopped with $150 \mu\text{l}$ of HClO_4 , and 30 nCi of cyclic $[^3\text{H}]\text{AMP}$ (internal standard) were added. Separation and counting of the product followed the two-column method of White & Karr (1978). Protein was measured by the method of Hartree (1972), using bovine serum albumin as standard.

RESULTS

Solulan activates the olfactory adenylate cyclase (Fig. 1), which remains responsive to guanine nucleotide analogues, to forskolin and to AlF_4^- . At very low detergent concentrations, the activity is still stimulated by odour (in the presence of GTP), but this response is lost at, or just below, the activating concentration of detergent.

The activation occurs over a very narrow range of detergent-to-protein (or detergent-to-lipid) ratios (Fig. 2), above which there is no further activation. Activation occurs at a Solulan concentration well below that necessary for any significant solubilization of the adenylate cyclase: 1–5% Solulan (detergent/protein $> 80 \mu\text{g}/\mu\text{g}$) solubilizes only about 10% of the activity in the absence of other disrupting factors, and such concentrations do not enhance activity beyond the levels shown in Fig. 1 (C. J. Robinson, S. G. Shirley & G. H. Dodd, unpublished work). This initial enhancement of cyclase activity slowly declines, the rate depending on temperature and detergent concentration, but the enzyme remains responsive to guanine nucleotides.

Abbreviations used: $\text{GDP}\beta\text{S}$, guanosine 5'-O-(2-thiodiphosphate); GPPNP, guanosine,5'-O- $[\beta\gamma\text{-imido}]$ triphosphate; $\text{GTP}\gamma\text{S}$, guanosine 5'-O-(3-thiotriphosphate); G-protein, guanine nucleotide binding protein.

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Fig. 1. Activation of olfactory adenylate cyclase by Solulan, showing the effects of different modulators

Reagent concentrations were: GTP γ S, 10 μ M; GPPNP, 10 μ M; GTP, 40 μ M; GDP β S, 10 μ M; forskolin 20 μ M; Al $^{3+}$, 20 μ M, F $^{-}$, 5 mM. The protein concentration was 0.1 μ g/ μ l. Odour activation is shown as (activity with 40 μ M-GTP + 1 mM acetophenone)/(activity with 40 μ M-GTP) $\times$ 100%; both being measured at the same Solulan concentration. The cyclase activity in the absence of Solulan is the same as that at the lowest concentration of Solulan shown. As the range of activities shown in the Figure is rather large, some of the numerical values are also given in Table 2. Data shown are from a single experiment, representative of four, and the duplicate data points showed a coefficient of variation of 2%.

The effect of Solulan is not reversible if the concentration is reduced by dilution, or by centrifugation and resuspension of the pellet in detergent-free buffer (Table 1). The cyclase activity of the membranes remains elevated and odour modulation is not re-established. Membranes pretreated with Solulan show higher cyclase activity than untreated membranes, but the activity is lower than that of untreated membranes assayed in the same concentration of Solulan (Table 1). This effect is more marked at higher detergent concentrations. The supernatant from pretreated and centrifuged membranes (containing detergent) has no effect on untreated membranes, other than that attributable to the detergent itself.

The Solulan-induced activation of the cyclase system is highly synergistic with that of GTP γ S, GPPNP, forskolin and AlF $_4^{-}$ (Fig. 1 and Table 2). Solulan affects the stimulation by GTP γ S by shifting the concentration of nucleotide necessary for half-maximal activation by more than one order of magnitude (Fig. 3). A similar finding with another detergent has been recently reported by Anholt (1988). Both with and without Solulan, the curves

Fig. 2. Activation of the olfactory adenylate cyclase by Solulan is dependent on the detergent/protein ratio

○, 0.0015% Solulan (w/v); ●, 0.005% Solulan; □, 0.015% Solulan. The activity is assayed in the presence of 10 μ M-GTP γ S. Data shown are duplicate determinations from a single experiment, representative of three.

Table 1. Reversibility of the effects of Solulan

An olfactory membrane suspension was incubated at 20 °C for 1 h in 0, 0.0128 or 0.128% Solulan in phosphate-buffered saline. The membranes were centrifuged and resuspended twice such that Solulan from the pretreatment was diluted at least 10 5 -fold in the assay. Concentrations of assay components were: GTP, 40 μ M; GTP γ S, 10 μ M; acetophenone (ace), 1 mM. Activities are the means of duplicate determinations.

Pretreatment	Components added	Adenylate cyclase activity (pmol/min per μ g of protein)	
		0% Solulan	0.0128% Solulan
None	GTP	0.97	30.24
	GTP + ace	2.33	28.82
	GTP γ S	7.31	66.39
0.0128% Solulan (2.43 μ g/ μ g of protein)	GTP	18.29	16.70
	GTP + ace	17.69	15.63
	GTP γ S	34.40	38.13
0.128% Solulan (24.3 μ g/ μ g of protein)	GTP	5.16	4.73
	GTP + ace	4.93	4.45
	GTP γ S	10.80	11.61

Table 2. Synergism in action of Solulan and other activators of adenylate cyclase

The data for 0.0016% Solulan and 0.0128% Solulan are extracted from Fig. 1. These are the points immediately to the left and right of the rising part of the curves. The data for zero Solulan cannot be plotted on the logarithmic scale of Fig. 1. Activities are the mean of duplicate determinations. Detergent/protein ratios ($\mu\text{g}/\mu\text{g}$) were 0 (0% Solulan), 0.17 (0.0016% Solulan) and 1.4 (0.0128% Solulan).

Activator	Solulan concentration (w/v)...	Adenylate cyclase activity (pmol of cyclic AMP/min per μg of protein)		
		0	0.0016%	0.0128%
GTP (40 μM)		0.96	0.77	22
GTP + ace (40 μM /1 mM)		2.9	1.46	22
		(+ 190 %)	(+ 90 %)	(- 2 %)
GTP γ S (10 μM)		6.8	5.9	56
GPPNP (10 μM)		6.5	6.1	54
Forskolin (20 μM)		7.2	8.4	68
Al ³⁺ (20 μM)/F ⁻ (5 mM)		4.0	5.6	56
GDP β S (10 μM)		0.40	0.58	17.2

Fig. 3. Activation of adenylate cyclase by GTP γ S in the presence or absence of Solulan

The arrows show the GTP γ S concentration at which half-maximal stimulation occurs. Protein concentration is 0.1 $\mu\text{g}/\mu\text{l}$. Data shown are duplicate determinations from a single experiment, representative of three.

are simple sigmoids, indicating one dissociation event. It should be noted that the highest GTP γ S concentrations are sufficient to compete with ATP for the Mg²⁺.

GTP γ S can activate the cyclase system both temporarily and permanently (Smigel *et al.*, 1982). In the

Table 3. Reversibility of activation at low concentrations of GTP γ S

Olfactory membranes were incubated for 1 h at 21 °C in phosphate-buffered saline, with or without GTP γ S. After centrifugation and resuspension, the estimated residual concentrations of GTP γ S in the assay were < 2 pM from the 20 nM pretreatment, and < 1 nM from the 10 μM pretreatment. Protein concentration was 0.07 $\mu\text{g}/\mu\text{l}$, and activities are the means of duplicate determinations.

Pretreatment	Assay	Adenylate cyclase activity (pmol of cyclic AMP/min per μg of protein)
-	-	0.35
20 nM-GTP γ S	20 nM-GTP γ S	4.0
	-	0.41
10 μM -GTP γ S	20 nM-GTP γ S	3.6
	-	4.7
	10 μM -GTP γ S	6.0

Table 4. The effect of pertussis-toxin-catalysed ribosylation on olfactory adenylate cyclase

Membranes (about 500 μg of protein/ml) were incubated with activated pertussis toxin (50 $\mu\text{g}/\text{ml}$) in 50 mM-sodium phosphate, 10 mM-thymidine, 10 mM-ATP, 1 mM-GTP, 10 mM-MgCl₂, 1 mM-EDTA, 2 mM-dithiothreitol, 5 mM-phosphoenolpyruvate, 20 μg of pyruvate kinase/ml and 50 μM -NAD⁺ at pH 6.8 for 30 min at 20 °C. For the controls, 'PBS' refers to membranes held in phosphate-buffered saline for 30 min at 30 °C, and 'buffer' refers to membranes held in ribosylation buffer without pertussis toxin for 30 min at 30 °C. The protein concentration is that measured before the ribosylation procedure. 'No guanine nucleotide' means that no nucleotide was added to the well-washed preparations, but no steps were taken to remove chemically very-tightly-bound endogenous guanine nucleotides. Activities are the mean of duplicate determinations.

Assay condition	Treatment...	Adenylate cyclase activity (pmol of cyclic AMP/min per μg of protein)		
		PBS	Buffer	Pertussis toxin
No guanine nucleotide		0.43	0.32	0.27
GTP (40 μM)		0.72	0.49	0.43
GTP (40 μM) + acetophenone (1 mM)		1.76	1.41	1.15
GDP β S (20 μM)		0.36	0.22	0.22
GTP γ S (20 μM)		4.7	3.9	3.5
GTP (40 μM) + Solulan (0.025%)		8.7	6.3	5.5
GDP β S (20 μM) + Solulan (0.025%)		9.5	6.8	5.4
GTP γ S (20 μM) + Solulan (0.025%)		28	21	15.8

Fig. 4. In Solulan, GTP γ S is competitive with ATP

○, No Solulan, 10 μ M-GTP γ S, plotted at $10 \times (1/\text{rate})$ for clarity; ▼, 0.05% Solulan + 10 μ M-GTP γ S; ▲, 0.05% Solulan + 200 nM-GTP α S; ▽, 0.05% Solulan + 20 nM-GTP γ S; △, 0.05% Solulan. Protein concentration is 0.1 μ g/ μ l; free Mg^{2+} concentration is 2 mM. Data shown are duplicate determinations from a single experiment, representative of two.

absence of Solulan, low (20 nM) concentrations of GTP γ S cause little permanent activation after 1 h incubation at 21 °C, though the temporary activation is easily visible. High (10 μ M) concentrations of GTP γ S cause permanent activation (Table 3).

In the presence of Solulan, GDP β S induces cyclase activities slightly higher than those obtained in the absence of guanine nucleotides (Table 4).

Treatment of the membranes with pertussis toxin and NAD results in a preparation whose response to odorant, nucleotides and detergent is very little changed (Table 4).

In Solulan, the cyclase activity is inhibited by ATP. This inhibition is reversed by GTP γ S. In the absence of Solulan, there is no such inhibition (Fig. 4).

DISCUSSION

In many systems, receptors are coupled to effector molecules via a guanine-nucleotide-binding protein (G-protein) which is either stimulatory (G_s) or inhibitory (G_i). In activated (GTP) form, the G-protein stimulates or inhibits the effector. Hydrolysis returns the G-protein to its non-activated (GDP) form [reviewed by Gilman (1987); Levitzki (1987)]. The precise mechanism of signal transduction by G-proteins is unknown.

Forskolin is believed to act on the cyclase catalytic unit, with its effect potentiated by activated G_s (Darfler *et al.*, 1982). GTP α S, GPPNP and AlF_4^- act on the

G-protein. Synergism between Solulan and these compounds suggests that the detergent has a different site of action. Moreover, the maximum activity induced by Solulan is greater than that evoked by the other stimulants.

It is unlikely that Solulan simply removes a barrier restricting ATP supply to the catalytic unit. With ATP supply limiting, odour and nucleotides could cause little change in activity. If a barrier were lifted, the higher activities should increase proportionally more than the lower ones, but the reverse was observed.

Solulan, despite its cholesterol head group, does not mimic the action of cholesterol. The variation of cholesterol/phospholipid molar ratios over a wide range has little effect on basal cyclase levels, and alters stimulated activities by a factor of only about 2 (Houslay & Gordon, 1983). We have found that a cholesterol suspension stimulates olfactory cyclase by 30%; but Solulan stimulates it by 10–40-fold (while less than stoichiometric with membrane phospholipids). This increase is too large to be an odorant stimulation by the cetyl alcohol component of Solulan.

The sharp detergent-activation curve (Hill coefficient of about 2) implies a more specific mechanism of action. 'Substrate' inhibition by ATP and its relief by GTP γ S suggests that Solulan may relax the specificity of the G-protein nucleotide binding site, allowing competition between the nucleotides. Changes in affinity of this site caused by Mg^{2+} (Salomon *et al.*, 1975) and Cl^- (Higashijima *et al.*, 1987) have been observed. While such competition could explain some (but by no means all) of the shift in k_{act} for GTP γ S, it would be inhibitory to the cyclase. The two phenomena might share a common cause, but activation proceeds despite the shift in k_{act} .

Frog olfactory cyclase has a k_{act} of about 1 nM (Pace & Lancet, 1986). In the absence of detergent, we find a value of 9 nM in the rat. These values are extremely low compared with the K_d of 0.7 μ M measured by the binding of GTP γ S to the G-protein of hepatic adenylate cyclase (Northup *et al.*, 1982). This could indicate an unusual G-protein or a stoichiometric excess of G-protein over adenylate cyclase.

GTP γ S can activate G-proteins both temporarily and permanently (Smigel *et al.*, 1982). A concentration of 10 μ M-GTP γ S in the cyclase assay gives maximal activity; pretreatment with this concentration gives a very noticeable permanent activation (Table 3). A concentration of 20 nM-GTP γ S in the assay induces more than half-maximal activity, but pretreatment with this concentration of nucleotide results in little permanent activation of the enzyme. Thus, the rate of formation of permanently-activated G-protein continues to increase manyfold after the GTP γ S concentration is already sufficient to produce near-maximum cyclase activity. This implies a stoichiometric excess of G-protein.

In this situation, Solulan could achieve cyclase activation (preserving G-protein nucleotide response), while increasing k_{act} , by increasing the size of the available pool of cyclase catalytic unit. k_{act} should increase by about the same factor as does basal cyclase activity; we observe 1–1.5 orders of magnitude for each increase. This implies that > 90% of the cyclase is normally silent and that Solulan releases this pool. This could indicate a second control point in the system. The loss of odour sensitivity could be connected with these phenomena or coincidental.

Could a second control site involve G_i ? Genes for several G-proteins are found in olfactory tissue (Jones & Reed, 1987) and one of these is a novel species. However, olfactory cyclase is insensitive to Na^+ (C. J. Robinson, S. G. Shirley & G. H. Dodd, unpublished work) and has a high (0.1 mM) optimum concentration for GTP (S. G. Shirley, C. J. Robinson & G. H. Dodd, unpublished work), which argue against a conventional dual-regulated system (Cooper, 1983). Also, pertussis-toxin-catalysed ribosylation does not grossly affect system function (Table 4; Pace & Lancet, 1986) although with such a high level of inhibition this might not be conclusive.

If the detergent over-rides an inhibition which is larger than the normal modulation of cyclase activity, then results from experiments employing detergent solubilization may need re-evaluation.

Solulan, at very low concentrations, has an effect on the olfactory cyclase activity which is not due to solubilization. This has proved useful in revealing some properties of the olfactory cyclase and G-protein.

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REFERENCES

- Anholt, R. R. H. (1988) *Biochemistry* **27**, 6464–6468
Cooper, D. M. F. (1983) *Curr. Top. Membr. Transp.* **18**, 67–84
Darfler, F. J., Mahan, L. C., Kochman, A. M. & Insel, P. A. (1982) *J. Biol. Chem.* **257**, 11901–11907
Gilman, A. G. (1987) *Annu. Rev. Biochem.* **56**, 615–649
Hartree, E. F. (1972) *Anal. Biochem.* **48**, 422–427
Higashijima, T., Ferguson, K. M. & Sternweiss, P. C. (1987) *J. Biol. Chem.* **262**, 3597–3602
Houslay, M. D. & Gordon, L. M. (1983) *Current Top. Membr. Transp.* **18**, 179–231
Johnson, R. A. & Sutherland, E. W. (1973) *J. Biol. Chem.* **248**, 5114–5121
Jones, D. T. & Reed, R. R. (1987) *J. Biol. Chem.* **262**, 14241–14249
Levitzki, A. (1987) *FEBS Lett.* **211**, 113–118
McDonald, I. A. (1975) *Biochim. Biophys. Acta* **397**, 244–253
Neer, E. J. (1978) *Adv. Cyclic Nucleotide Res.* **9**, 69–83
Northup, J. K., Smigel, M. D. & Gilman, A. G. (1982) *J. Biol. Chem.* **257**, 11416–11423
Pace, U. & Lancet, D. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 4947–4951
Perkins, J. A. & Moore, M. M. (1971) *J. Biol. Chem.* **246**, 62–68
Salomon, Y., Lin, M. C., Londos, C., Rendell, M. & Rodbell, M. (1975) *J. Biol. Chem.* **250**, 4239–4245
Shirley, S. G., Robinson, C. J., Dickinson, K., Aujla, R. & Dodd, G. H. (1986) *Biochem. J.* **240**, 605–607
Shirley, S. G., Robinson, C. J. & Dodd, G. H. (1987) *Biochem. Soc. Trans.* **15**, 502
Smigel, M. D., Northup, J. K. & Gilman, A. G. (1982) *Recent Prog. Horm. Res.* **38**, 601–624
Sutherland, E. W., Rall, T. W. & Menon, T. (1962) *J. Biol. Chem.* **237**, 1220–1227
White, A. A. & Karr, D. B. (1978) *Anal. Biochem.* **85**, 451–460

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