The activity of dopamine-stimulated adenylate cyclase from rat brain striatum is modulated by temperature and the bilayer-fluidizing agent, benzyl alcohol

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Benzyl alcohol achieved a marked activation of the adenylate cyclase activity in a partially purified membrane preparation from rat brain striata, although inhibition resulted at high concentrations. The degree of activation observed depended on the ligand used to stimulate the enzyme, with that observed in the presence of guanosine 5'-[β , γ -imido]triphosphate (p[NH]ppG) (5.8-fold) > dopamine + p[NH]ppG (5-fold) > GTP (3-fold) > dopamine + high GTP (2.25-fold) > dopamine (+ low GTP) = basal (+ low GTP) (1.7-fold). The differences in the concentration-dependence of both the activation and inhibition of dopamine-stimulated and basal activities of the enzyme meant that increasing benzyl alcohol concentrations caused a net elevation in the fold-stimulation of the basal activity by dopamine. Arrhenius plots of p[NH]ppG-, GTP-, fluoride-, dopamine-plus-high GTP- and dopamine-plus-p[NH]ppG-stimulated activities all exhibited a single break occurring at around 22°C. This break point was decreased to around 13°C when 50 mM-benzyl alcohol was added to the assays. In the presence of dopamine (+ low GTP), Arrhenius plots exhibited two distinct breaks, one at around 21°C and the other at around 11°C. When benzyl alcohol (50 mM) was added to these assays of dopamine (+ low GTP)-stimulated activity, a single break at around 14°C was observed. For the basal activity the Arrhenius plot exhibited a single break at around 15°C both in the presence and in the absence of 50 mM-benzyl alcohol. It is suggested that the enzyme is activated by productive collisions between independent mobile entities and that the activity of the enzyme may be regulated by changes in membrane fluidity. The breaks in the Arrhenius plots of all of the ligand-stimulated activities, but not the basal activity, are attributed to lipid-phase separations occurring in either the inner or the outer halves of the bilayer.

A variety of physiological functions of dopamine in the central nervous system are mediated by dopamine binding to specific receptors, which are linked to an adenylate cyclase system (Kebabian *et al.*, 1972; Miller *et al.*, 1974). The adenylate cyclase-linked dopamine receptor, referred to as the D₁-receptor (Zahnier & Molinoff, 1978), is regulated by guanine nucleotides and is found in high amounts in rat brain corpus striatum (Creese *et al.*, 1979). Striatal homogenates are thus a good source of adenylate cyclase activity, which is actively stimulated by dopamine and guanine nucleotides (Clement-Cormier *et al.*, 1975; McSwigan *et al.*, 1980). This

system has proved to be a good model for investigating the action and potency of neuroleptic drugs (Miller *et al.*, 1974; Iversen *et al.*, 1976).

Both the dopamine receptor and adenylate cyclase in brain are integral membrane proteins that can be solubilized with appropriate detergents (Gorissen & Laduron, 1979; Gorissen *et al.*, 1979; Hoffman, 1979; Sano *et al.*, 1979). It has been reported that dopamine-stimulated activity can be reconstituted on mixing of isolated receptor and adenylate cyclase components (Hoffman, 1979; Sano *et al.*, 1979) and that phospholipids are essential for this process (Hoffman, 1979) and for regulating the activity of the enzyme (Hebdon *et al.*, 1979).

The activity of a wide variety of integral membrane enzymes is affected by alterations in lipid

Abbreviation used: p[NH]ppG, guanosine 5'-[β , γ -imido]triphosphate.

fluidity (see Kimelberg, 1977). Indeed, alterations in membrane lipid fluidity have been shown to modulate the activity of adenylate cyclases interacting with the catecholamine receptor (Orly & Schramm, 1975; Rimón *et al.*, 1978; Barkardjieva *et al.*, 1979), glucagon receptor (Dipple & Houslay, 1978; Houslay *et al.*, 1981; Houslay & Gordon, 1982) and prostaglandin E_1 receptor (Sinensky *et al.*, 1979; Brasitus & Schachter, 1980). Changes in membrane fluidity are caused by a wide variety of xenobiotics and also occur during transformation, aging and in various disease states (see Kimelberg, 1977). It is thus of some importance to ascertain the effects of alterations in membrane fluidity and sensitivity to the membrane environment of key regulatory enzymes. In the present study we examine the effect of the neutral local anaesthetic and bilayer-fluidizing agent, benzyl alcohol (Colley & Metcalfe, 1972; Gordon *et al.*, 1980) on the activity of the dopamine-stimulated adenylate cyclase from rat striata and examine the thermodependence of the activities of this enzyme.

Materials and methods

Male Sprague-Dawley rats (200–300 g) were killed by cervical dislocation. The striata were removed as described by Kebabian *et al.* (1972), washed in homogenizing buffer (2 mM-EGTA/2 mM-Tris/maleate, final pH 7.4) and then homogenized in a glass/Teflon homogenizer at 400 rev./min with eight strokes with 50 vol. of homogenizing buffer. The resultant homogenate was fractionated by using a modification of that described by Sieghart *et al.* (1976). Briefly, it was centrifuged for 10 min at 1000 *g* and the resultant supernatant taken and re-centrifuged at 10000 *g* for 20 min. The pellet produced was resuspended in the original volume of homogenizing buffer and re-centrifuged at 10000 *g* for 20 min. This washing procedure was repeated twice before the pellet was finally resuspended in 25% of the original homogenate volume of homogenizing buffer. This fraction could be stored for several months at -80°C without changes in the various adenylate cyclase activities compared with fresh material.

The adenylate cyclase assay was a modification of that described by Clement-Cormier *et al.* (1975). The standard assay conditions used contained final concentrations of 0.2 mM-EGTA, 10 mM-theophylline, 2 mM- MgSO_4 , 0.5 mM-ATP, 7.4 mg of phosphocreatine/ml, 1 mg of creatine kinase/ml and 50 mM-Tris/maleate, final pH 7.2. Ligands and benzyl alcohol were added at the concentrations described in the text and reactions were initiated with the membrane fraction (final assay concentration, 80–140 μg of protein/ml). Incubations were carried out at 30°C , unless otherwise stated, and

initial rates taken from linear time courses. Reactions were terminated by boiling the mixtures for 3 min and cyclic AMP was determined by a modification of the method of Brown *et al.* (1972) as described previously (Houslay *et al.*, 1976). The addition of benzyl alcohol did not affect this assay of cyclic AMP. To retain dopamine sensitivity it was essential to include EGTA in both the homogenization buffers and subsequent assay mixtures. This is in agreement with the observations of Clement-Cormier *et al.* (1975).

Analysis using Arrhenius plots utilized initial rates from linear time courses over the entire temperature range studied. Break points in these plots were determined by using a least-squares minimalization procedure that has been described in some detail previously (Houslay & Palmer, 1978).

Creatine kinase, ATP, cyclic AMP, GTP, p[NH]ppG and triethanolamine/HCl were from Boehringer (U.K.), Lewes, East Sussex, U.K. Phosphocreatine and dopamine were from Sigma (London) Chemical Co., Kingston upon Thames, Surrey, U.K. [^3H]Cyclic AMP was from The Radiochemical Centre, Amersham, Bucks., U.K. All other chemicals were of A.R. grade from BDH Chemicals, Poole, Dorset, U.K.

Results

As McSwigan *et al.* (1980) reported for the activity of rat striatal adenylate cyclase in a crude homogenate, we, using a washed partially purified membrane fraction, obtained similar results for the stimulation of basal (+50 nM-GTP) activity (100%) by 10 μM -GTP (160%), 10 μM -p[NH]ppG (280%), 15 mM-fluoride (246%), 0.1 mM-dopamine + (low) 50 nM-GTP (160%), 0.1 mM-dopamine + (high) 10 μM -GTP (328%) and 0.1 mM-dopamine + 10 μM -p[NH]ppG (384%). These observations confirm the role of guanine nucleotides in activating this enzyme as reported by McSwigan *et al.* (1980), which is in line with their effects on adenylate cyclase from many other sources (see Ross & Gilman, 1979). In contrast with the results of Clement-Cormier *et al.* (1975) we did not lose dopamine-stimulated activity during the preparation of a membrane fraction from the homogenate. However, in their fractionation study they used media containing sucrose, which one of us (L. Needham, unpublished work) has found to be responsible for destroying the dopamine response.

The low GTP level (50 nM) stated reflects the amount of GTP present in our ATP preparation (see Houslay & Gordon, 1982). This has been shown previously by us to allow activation of liver plasma-membrane systems by glucagon to occur, although saturating (high) GTP concentrations (10 μM) not

Fig. 1. Sensitivity of striatal adenylate cyclase to benzyl alcohol

(a) Basal (▲) and GTP- (■) and p[NH]ppG-stimulated (●) adenylate cyclase activities; (b) dopamine (+ low GTP)- (Δ), dopamine (+ high GTP)- (□) and dopamine (+ p[NH]ppG)-stimulated (○) adenylate cyclase (c) Stimulation ratio (fold) achieved by dopamine. Δ, Dopamine (+low GTP)/basal (+low GTP) activities; □, dopamine (+high GTP)/(+high GTP) activities; ○, dopamine (+p[NH]ppG)/p[NH]ppG activities. High GTP and p[NH]ppG were at final concentrations of 10 μ M and dopamine was at a final concentration of 0.1 mM. Low GTP concentrations were 50 nM. Assays were conducted at 30°C in all cases. In the basal state the enzyme exhibited a specific activity of 50.1 $\text{pmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$. Fold activation by ligands is given in the results section.

only increased the degree of activation but altered the coupling mechanism (see Houslay, 1981; Houslay & Gordon, 1982; Houslay *et al.*, 1980a; Martin *et al.*, 1979).

Increases in benzyl alcohol concentration led to a progressive augmentation of all of the various adenylate cyclase activities associated with the rat striatal membrane fraction used (Figs. 1a and 1b). In all cases an optimum concentration was reached, above which the activities were reduced. This optimum benzyl alcohol concentration appeared to be higher for the guanine nucleotide-stimulated states of the enzymes, which has been noted before for the liver system (Houslay *et al.*, 1981). The most marked effects were observed in the p[NH]ppG-stimulated states both with and without dopamine, where a 5–6-fold increase in activity ensued (Figs. 1a and 1b). If the dopamine-stimulation ratio was plotted as a function of benzyl alcohol concentration (Fig. 1c) then it can be seen that increasing benzyl alcohol concentrations causes a marked rise in the ratio of dopamine (plus low GTP) stimulation over the basal (plus low GTP) activity. However, benzyl alcohol has little effect on the degree of stimulation by dopamine plus high guanine nucleotide over the high guanine nucleotide-stimulated state alone (Fig. 1c).

A detailed Arrhenius plot analysis of the various ligand-stimulated and basal (plus low GTP) states, in the presence and absence of 50 mM-benzyl alcohol, was carried out. The results are summarized in Table 1. The data obtained were of the quality obtained by us in previous studies on other systems

(see Houslay & Palmer, 1978; Houslay *et al.*, 1980a) and were analysed as described previously in these publications. The changes in activity reported were fully reversible under the assay conditions used. We noted that the activity became extremely thermolabile at temperatures greater than 35°C, although guanine nucleotides appeared to afford some protection. As can be seen, the basal (+ low GTP) activity exhibited a single break at around 15°C both in the presence and in the absence of benzyl alcohol. In contrast, Arrhenius plots of p[NH]ppG-, high GTP-, fluoride-, dopamine-plus-p[NH]ppG- and dopamine-plus-high-GTP-stimulated activities all exhibit a single break at around 22°C that decreased to some 13°C when benzyl alcohol (50 mM) was present in the assay. Dopamine (+ low GTP)-stimulated adenylate cyclase, however, yielded Arrhenius plots of its activity that had two distinct breaks occurring at around 21°C and 11°C. The addition of 50 mM-benzyl alcohol to the assays resulted in Arrhenius plots displaying but a single break at around 14°C.

Discussion

Benzyl alcohol is a neutral molecule, which precludes any charge interaction between it and membrane components. It has been shown to effect marked increases in fluidity of both model (Colley & Metcalfe, 1972) and biological (Gordon *et al.*, 1980; Brasitus & Schachter, 1980) membranes and has been used successfully to determine the effect of increased bilayer fluidity on a variety of integral

Table 1. *Arrhenius plots of the various ligand-stimulated states of striatal adenylate cyclase activity: the effect of benzyl alcohol*

Errors given in parentheses are 95% confidence limits derived from two to three experiments carried out on the pooled striata from 18 rats. The break points and activation energies were derived by using a least-squares minimalization technique (Houslay & Palmer, 1978). Initial rate measurements were made over a temperature range of 2 to 35°C at between 23 and 30 different temperatures. Reversible changes in activity were noted under the incubation conditions used in this study. The quality of data obtained and form of the plots was similar to that obtained by us previously in other systems (see Houslay & Palmer, 1978; Houslay *et al.*, 1980a,b).

Stimulating ligand	Presence of 50 mM-benzyl alcohol	Break point (°C)	Activation energy (kJ/mol)		
			Above upper break	Below upper break	Below lower break
None (low, 50nM GTP)	No	15.8 (14.7–17.6)	6.2 (3.9–9.6)	100.5 (87.4–105)	
None (low GTP)	Yes	15.5 (13.5–17.1)	20.7 (18.0–29.3)	61.3 (55.6–61.9)	
p[NH]ppG	No	21.3 (20.0–22.7)	51.5 (48.1–58.2)	100.8 (97.9–101.3)	
p[NH]ppG	Yes	12.9 (12.4–14.4)	38.9 (40.0–43.6)	92.9 (84.1–93.3)	
GTP	No	22.0 (19.0–23.0)	34.1 (33.6–36.7)	56.9 (55.6–59.1)	
GTP	Yes	13.1 (11.9–14.8)	44.2 (43.0–46.4)	71.0 (62.3–75.3)	
Fluoride	No	22.1 (21.1–22.7)	7.5 (5.0–12.1)	57.3 (54.0–59.4)	
Fluoride	Yes	11.4 (10.1–11.9)	60.8 (58.1–61.5)	94.1 (88.3–98.7)	
Dopamine (+ low GTP)	No	21.0 (upper) (19.6–23.3) 10.9 (lower) (10.2–11.9)	3.85 (1.84–8.8)	45.8 (29.7–54.4)	85.4 (79.9–91.2)
Dopamine (+ low GTP)	Yes	14.3 (13.2–15.6)	15.6 (12.6–22.4)	79.8 (75.6–81.7)	
Dopamine + p[NH]ppG	No	22.5 (21.6–23.9)	34.3 (33.8–37.1)	63.0 (59.4–64.4)	
Dopamine + p[NH]ppG	Yes	14.7 (13.2–16.7)	8.5 (2.8–16.3)	126.4 (109.4–133.6)	
Dopamine + GTP	No	23.7 (21.0–26.5)	36.4 (35.9–39.3)	52.3 (44.4–53.1)	
Dopamine + GTP	Yes	15.0 (13.2–15.9)	49.2 (48.6–51.2)	92.2 (86.9–102.8)	

membrane enzymes (Houslay *et al.*, 1978; Gordon *et al.*, 1980), including adenylate cyclase (Dipple & Houslay, 1978; Brasitus & Schachter, 1980; Gordon *et al.*, 1980; Amir *et al.*, 1981; Krall *et al.*, 1981). In all of these instances, the increases in bilayer fluidity achieved by benzyl alcohol led to the activation of the enzyme (see Houslay & Gordon, 1982, for review). This is consistent with the effects of other agents that increase bilayer fluidity and also lead to rises in adenylate cyclase activity such as cationic (Houslay *et al.*, 1980b) and anionic (Houslay *et al.*, 1981) local anaesthetics as well as fatty acids (Orly & Schramm, 1975). On the other hand, elevated cholesterol levels, which cause a decrease in bilayer fluidity, lead to a corresponding reduction

in adenylate cyclase activity (see Houslay & Gordon, 1982, for review).

Benzyl alcohol has marked activity effects on basal and ligand-stimulated adenylate cyclase activity (Fig. 1) over a concentration range where it has been shown both to increase bilayer fluidity in model and biological membrane systems (Colley & Metcalfe, 1972; Brasitus & Schachter, 1980; Gordon *et al.*, 1980) and to activate adenylate cyclase from other sources (Dipple & Houslay, 1978; Brasitus & Schachter, 1980). It is noteworthy that when high concentrations of guanine nucleotides, in particular the non-hydrolysable analogue of GTP, p[NH]ppG, are present in the assays then stimulation is greatest (Fig. 1), an observation

that has been noted in the liver system (Houslay *et al.*, 1980a). In all cases, high benzyl alcohol concentrations led to an inhibition of enzyme activity. This is analogous to the situation observed for many other types of enzymes and evidence has been presented to suggest that it may be due to benzyl alcohol competing for sites on the protein that are normally occupied by annular phospholipids (see Gordon *et al.*, 1980, for detailed discussion). The similarity of the response to benzyl alcohol of striatal adenylate cyclase and other integral membrane enzymes, including adenylate cyclase from other sources, would imply that the activity of this enzyme is markedly affected by increases in bilayer fluidity. To gain support for this contention we examined the thermodependence of the various ligand-stimulated activities. This is because many enzymes whose activities are sensitive to changes in the physical properties of their lipid environment exhibits breaks in Arrhenius plots of their activities that coincide with lipid-phase separations occurring in the bilayer (see, e.g., Melchior & Steim, 1976; Kimelberg, 1977; Houslay & Palmer, 1978; Sander-mann, 1978). Furthermore it has been demonstrated that benzyl alcohol can decrease the temperature at which such lipid-phase separations occur by some 6–10°C (Colley & Metcalfe, 1972; Dipple & Houslay, 1978; Houslay & Palmer, 1978; Brasitus & Schachter, 1980; Gordon *et al.*, 1980) as it is excluded from gel-phase lipid and acts as an impurity, thus depressing the freezing point (Hill, 1974; Lee, 1977). As we can see (Table 1), Arrhenius plots of all the ligand-stimulated activities, except for that where dopamine (+low GTP) is present, exhibit a single break at around 22°C that is decreased by 9°C to around 13°C in the presence of benzyl alcohol. This is strong evidence that the break at 22°C is due to a lipid-phase separation occurring at this temperature in the membrane, whose onset is depressed to 13°C by benzyl alcohol. Such an effect on the Arrhenius plots of enzymes whose activities are affected by lipid-phase separations has been noted for adenylate cyclase and a variety of other enzymes in tissues from various sources (Dipple & Houslay, 1978; Houslay & Palmer, 1978; Brasitus & Schachter, 1980). In these instances thermotropic lipid phase separations have also been identified and characterized using physical probes (Houslay & Palmer, 1978; Brasitus & Schachter, 1980; Gordon *et al.*, 1980). Unfortunately, the inability to fractionate our partially purified membrane preparation by standard techniques owing to enzyme inactivation (see Clement-Cormier *et al.*, 1975; see also the Results section) prevents any detailed physical investigation at present. In contrast, the dopamine (+low GTP)-stimulated activity yielded Arrhenius plots with two distinct breaks, one at around 21°C and the other at around 11°C.

Like the other ligand-stimulated activities that sensed the high temperature break point, this too was decreased to around 13–14°C in the presence of 50 mM-benzyl alcohol, indicating a common sensitivity to presumably a lipid-phase separation occurring at around 22°C. The lower break point at around 11°C experienced by the dopamine (+low GTP)-stimulated activity may also be due to a lipid-phase separation as it was apparently abolished. Such a lipid-phase separation would presumably be decreased by benzyl alcohol to a temperature below which we could accurately detect it (<4°C). Arrhenius plots of the basal (+low GTP) enzyme activity, however, are biphasic with a break occurring at around 15°C, which is not apparently affected by the presence of 50 mM-benzyl alcohol. This would preclude the break being a lipid-mediated effect. There are many other mechanisms that could give rise to biphasic Arrhenius plots (see Han, 1972) and our observations imply that this break point reflects a protein-mediated event. Indeed, the basal (+low GTP) adenylate cyclase activity from a number of sources exhibits a break point that is apparently insensitive to the presence of bilayer-fluidizing agents, which would decrease the temperature at which the lipid-phase separation occurs (see Orly & Schramm, 1975; Brasitus & Schachter, 1980; Houslay & Gordon, 1982). It has been suggested (Houslay & Gordon, 1982) that the break in the Arrhenius plot of the basal (+low GTP) activity reflects the equilibrium between two different activity states of the enzyme, whereas one state entirely predominates in the presence of activating ligands (Birnbaumer *et al.*, 1980), whose reactions would presumably exhibit different activation energies. In this instance the effect is so dramatic that it appears to dominate any influence that the lipid-phase separation might be expected to have on the form of the Arrhenius plot in this state of the enzyme.

This precise pattern of sensitivities of the different ligand-stimulated adenylate cyclases to lipid-phase separations occurring in the membranes has been observed for the glucagon-stimulated enzyme in both hamster and rat liver plasma membranes (Houslay, 1981; Houslay *et al.*, 1980a). In these instances it has been suggested first that, in the absence of hormone, the receptor is not associated with the enzyme and secondly that the fluoride- and guanine nucleotide-stimulated states of the enzyme, which reflect interactions between the G-protein and catalytic unit exposed at the cytosol surface of the membrane (Ross & Gilman, 1979; Limbird, 1981), only experience the lipid environment of the inner cytosol-facing half of the bilayer (Houslay *et al.*, 1980a, 1981; Houslay, 1981; Houslay & Gordon, 1982). However, in the presence of hormone (+low GTP) alone, evidence from a number of independent approaches (Houslay *et al.*,

1977, 1980a; Martin *et al.*, 1979; Schramm, 1979; Houslay, 1981) has shown that the receptor, with its binding site on the cell surface, interacts to form a transmembrane complex with the G-protein and the catalytic unit by a Mobile Receptor Model (Houslay, 1981). This complex with the receptor conveys sensitivity to the outer half of the bilayer on the enzyme (see Houslay, 1981; Houslay & Gordon, 1982). The presence of saturating (high) concentrations of guanine nucleotides together with the hormone initiates a Collision Coupling Mechanism (Tolkovsky & Levitzki, 1978; Houslay *et al.*, 1980) whereupon the complex of receptor, G-protein and catalytic unit dissociates to yield an active catalytic unit, whose steady-state activity only senses the inner half of the bilayer (Houslay, 1981; Houslay *et al.*, 1980). The striking similarity of the results observed here to those of the glucagon systems in rat and hamster liver plasma membranes lead us to suggest that dopamine activates adenylate cyclase in the striatum by similar means. On this basis we would like to attribute the break at around 22°C to a lipid-phase separation occurring in the inner (cytosol) half of the bilayer as it is experienced by all the ligand-stimulated activities. In the presence of dopamine (+low GTP) the Arrhenius plot exhibits a sensitivity not only to this break at 22°C, but also to a break at around 11°C, which, by its sensitivity to benzyl alcohol, is also presumably due to a lipid-phase separation. At such low concentrations of GTP, the enzyme in liver plasma membranes forms a transmembrane complex with the receptor (see Houslay, 1981). On this basis we would like to attribute the break at around 11°C to a lipid-phase separation occurring in the external half of the bilayer which only affects the enzyme when it is obeying a Mobile Receptor Mechanism (see Houslay, 1981; Houslay & Gordon, 1982). We consider that this is by far a more satisfactory explanation than the possibility that under such conditions the activity measured represents a mixture of enzyme species in both the basal (+low GTP) and dopamine (+high GTP)-stimulated states. If this were true then we would expect to see an Arrhenius plot with breaks occurring at 24°C and 16°C instead of at 21°C and 11°C (Table 1). Furthermore, in rat liver plasma membranes we would expect by such an argument to observe a single break at 22°C that is insensitive to benzyl alcohol rather than a single break at 28°C that is sensitive to benzyl alcohol (see Houslay *et al.*, 1980a, 1981; Houslay & Gordon, 1982) and in hamster liver plasma membranes a single break at 25–26°C rather than two breaks, one at 25°C and another at 12°C (Houslay & Palmer, 1978). Indeed our use of charged drugs (see Houslay *et al.*, 1981) for achieving perturbations of rat liver plasma membranes yields effects that are consistent with

this interpretation of the mechanism of activation of adenylate cyclase and the asymmetric orientation of the various components in the bilayer.

In both cases of the proposed lipid-phase separations occurring at around 22°C and at around 11°C, which have been tentatively attributed to the inner and outer halves of the bilayer respectively, the single breaks observed in Arrhenius plots of adenylate cyclase activity presumably reflect the high temperature onset or fluidus boundary of the lipid-phase separation. That a further break is not associated with each of these might well mean that the solidus boundary or completion of the lipid-phase separation occurs at a temperature that is below our detection limit (<4°C), as would appear to be the case in both rat and hamster liver plasma membranes (see Houslay & Gordon, 1982). It is, however, possible that a single break might be observed, even though the solidus boundary had been crossed, if the enzyme remained in a fluid microenvironment. However, this is perhaps rather unlikely, as one would have to hypothesize that the thermodynamic constraints on this pool of lipid would be identical at temperatures between the solidus and fluidus boundaries with those at temperatures below the solidus boundary.

On the basis of these results and observations that the dopamine receptor and adenylate cyclase are physically separate entities, we would like to suggest that dopamine activates adenylate cyclase through productive collisions between independent mobile entities as has been suggested for the β -receptor (Schramm *et al.*, 1977; Tolkovsky & Levitzki, 1978; Limbird *et al.*, 1979; Atlas *et al.*, 1980; DeLean *et al.*, 1980; Limbird, 1981) the glucagon receptor (Houslay *et al.*, 1977, 1980a; Schramm, 1979; Houslay, 1981), vasopressin receptor and neurohypophyseal receptor (Bergman & Hechter, 1978). Similarly, that both the activities of the enzyme and dopamine binding are regulated by guanine nucleotides (Creese *et al.*, 1979; McSwigan *et al.*, 1980) suggests that a guanine nucleotide (G) binding protein also regulates the activity of the enzyme and its response to dopamine. Depending on the variety and concentration of ligands present, the catalytic unit of adenylate cyclase will be in different activity states and may also form various complexes with and without the G-protein and dopamine receptor (see Houslay & Gordon, 1982). Thus it is not too surprising that striatal adenylate cyclase activity responses differently in its various ligand-stimulated states to benzyl alcohol. We can expect that the functioning of the dopamine receptor coupled to adenylate cyclase will be rather sensitive to perturbations of its membrane lipid environment that may be either caused by various drugs, anaesthetics or that occurs during aging, various disease states and transformation.

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References

- Amir, S. M., Mubrow, N. J. & Inbar, S. H. (1981) *Endocrine Res. Commun.* **8**, 83–95
- Atlas, D., Volsky, D. J. & Levitzki, A. (1980) *Biochem. Biophys. Acta* **597**, 64–69
- Barkardjieva, A., Galla, H. J. & Helmreich, E. J. M. (1979) *Biochemistry* **18**, 3016–3023
- Bergman, R. N. & Hechter, O. (1978) *J. Biol. Chem.* **253**, 3238–3250
- Birnbaumer, L., Bearer, C. F. & Iyengar, R. (1980) *J. Biol. Chem.* **255**, 3552–3557
- Brasitus, T. A. & Schachter, D. (1980) *Biochemistry* **18**, 2763–2769
- Brown, B. L., Ekins, R. P. & Albano, J. D. M. (1972) *Adv. Cyclic Nucleotide Res.* **2**, 25–40
- Clement-Cormier, Y. C., Parrish, R. G., Petzold, G. L., Kebabian, J. W. & Greengard, P. (1975) *J. Neurochem.* **25**, 143–149
- Colley, C. M. & Metcalfe, J. C. (1972) *FEBS Lett.* **24**, 241–246
- Creese, I., Usdin, T. & Synder, S. H. (1979) *Nature (London)* **278**, 577–578
- DeLean, A., Stadel, J. M. & Lefkowitz, R. J. (1980) *J. Biol. Chem.* **255**, 7108–7117
- Dipple, I. & Houslay, M. D. (1978) *Biochem. J.* **174**, 179–190
- Gordon, L. M., Sauerheber, R. D., Esgate, J. A., Dipple, I., Marchmont, R. J. & Houslay, M. D. (1980) *J. Biol. Chem.* **255**, 4519–4527
- Gorissen, H. & Laduron, P. (1979) *Nature (London)* **279**, 72–74
- Gorissen, H., Aerts, G. & Laduron, P. (1979) *FEBS Lett.* **100**, 281–286
- Han, M. H. (1972) *J. Theor. Biol.* **35**, 543–568
- Hebdon, G. H., Levine, H. III, Minard, R. B., Sayhoun, N. E., Schmitges, C. J. & Cuatrecasas, P. (1979) *J. Biol. Chem.* **254**, 10459–10465
- Hill, M. W. (1974) *Biochim. Biophys. Acta* **356**, 117–124
- Hoffman, F. M. (1979) *J. Biol. Chem.* **254**, 255–258
- Houslay, M. D. (1981) *Adv. Cyclic Nucleotide Res.* **14**, 111–119
- Houslay, M. D. & Gordon, L. M. (1982) *Curr. Top. Memb. Transp.* in the press
- Houslay, M. D. & Palmer, R. W. (1978) *Biochem. J.* **174**, 909–919
- Houslay, M. D., Metcalfe, J. C., Warren, G. B., Hesketh, T. R. & Smith, G. A. (1976) *Biochim. Biophys. Acta* **436**, 489–494
- Houslay, M. D., Ellory, J. C., Smith, G. A., Hesketh, T. R., Stein, J. M., Warren, G. B. & Metcalfe, J. C. (1977) *Biochim. Biophys. Acta* **467**, 208–219
- Houslay, M. D., Palmer, R. W. & Duncan, R. J. S. (1978) *J. Pharmac. Pharmacol.* **30**, 711–714
- Houslay, M. D., Dipple, I. & Elliott, K. R. F. (1980a) *Biochem. J.* **186**, 649–658
- Houslay, M. D., Dipple, I., Rawal, S., Sauerheber, R. D., Esgate, J. A. & Gordon, C. M. (1980b) *Biochem. J.* **190**, 131–137
- Houslay, M. D., Dipple, I. & Gordon, L. M. (1981) *Biochem. J.* **197**, 675–681
- Iversen, L. L., Rogawski, M. A. & Miller, R. J. (1976) *Mol. Pharmacol.* **12**, 251–262
- Kebabian, J. W., Petzold, G. L. & Greengard, P. (1972) *Proc. Natl. Acad. Sci. U.S.A.* **69**, 2145–2149
- Kimelberg, H. E. (1977) in *Dynamic Aspects of Cell Surface Organisation* (Poste, G. & Nicolson, G. L., eds.), pp. 205–293, Elsevier/North-Holland Biomedical Press, Amsterdam
- Krall, J. F., Leshon, S. C., Frolich, M. & Korenman, S. G. (1981) *J. Biol. Chem.* **256**, 5436–5442
- Lee, A. G. (1977) *Biochim. Biophys. Acta* **472**, 285–344
- Limbird, L. E. (1981) *Biochem. J.* **195**, 1–13
- Limbird, L. E., De Lean, A., Hickey, A. R., Pike, L. J. & Lefkowitz, R. J. (1979) *Biochim. Biophys. Acta* **586**, 298–314
- Martin, B. R., Stein, J. M., Kennedy, E. L., Doberska, C. A. & Metcalfe, J. C. (1979) *Biochem. J.* **184**, 253–260
- McSwigan, J. D., Nicol, S. E., Gottesman, I. I., Tuason, V. B. & Frey, W. H., II (1980) *J. Neurochem.* **34**, 594–601
- Melchior, D. L. & Steim, J. M. (1976) *Annu. Rev. Biophys. Bioeng.* **3**, 179–201
- Miller, R. J., Horn, A. S. & Iversen, L. L. (1974) *Mol. Pharmacol.* **10**, 759–766
- Orly, J. & Schramm, M. (1975) *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3433–3437
- Rimon, G., Hanski, E., Braun, S. & Levitzki, A. (1978) *Nature (London)* **276**, 394–396
- Ross, E. M. & Gilman, A. G. (1979) *Annu. Rev. Biochem.* **49**, 533–564
- Sandermann, H. (1978) *Biochim. Biophys. Acta* **515**, 209–237
- Sano, K., Nishikori, K., Noshiro, O. & Maeno, H. (1979) *Arch. Biochem. Biophys.* **197**, 285–293
- Schramm, M. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 1174–1178
- Schramm, M., Orly, J., Elmerl, S. & Korner, M. (1977) *Nature (London)* **268**, 210–313
- Sieghart, W., Leitch, H. & Karobath, M. (1976) *Brain Res.* **109**, 418–422
- Sinensky, M., Minneman, K. P. & Molinoff, P. (1979) *J. Biol. Chem.* **254**, 9135–9141
- Tolkovsky, A. M. & Levitzki, A. (1978) *Biochemistry* **17**, 3795–3810
- Zahnier, N. B. & Molinoff, P. B. (1978) *Nature (London)* **275**, 453–455