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Olfactory adenylate cyclase of the rat

Stimulation by odorants and inhibition by Ca^{2+}

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Membranes prepared from the olfactory mucosa of the rat show a high level of adenylate cyclase activity. The activity increases up to 2-fold in the presence of physiologically relevant concentrations of odorants and is inhibited by Ca^{2+}. The level of cyclase activity found is sufficient to explain the speed of olfactory transduction, which occurs on a time scale of tens of milliseconds.

INTRODUCTION

The possible involvement of cyclic AMP in olfactory transduction has long been suspected. Kurihara & Koyama (1972) demonstrated the presence of high levels of adenylate cyclase in the olfactory mucosa, but did not report any attempts to stimulate this activity with odours. Menevse *et al.* (1977) showed that cyclic AMP analogues and phosphodiesterase inhibitors affected the production of the EOG (electro-olfactogram) (an indicator of early electrical events in odour transduction).

Menevse *et al.* (1977) found that odorants did not stimulate the adenylate cyclase of olfactory membrane preparations. However, the details of their preparation were not given. In the present paper we report investigations of sonicated olfactory material from the rat which show the presence of an odour-stimulated adenylate cyclase.

Recently, an odour-modulated adenylate cyclase has been found in the frog (Pace *et al.*, 1985; Anholt, 1986). This seems to have properties very similar to the rat tissue preparation described here.

MATERIALS AND METHODS

Animals

Male Wistar rats of about 300 g body weight were used.

Chemicals

The radiolabelled biochemicals were supplied by Amersham International; all other biochemicals were from Sigma. Chemicals, except imidazole, were of analytical quality. Odorants were of the highest commercially available quality and were used without further purification.

Adenylate cyclase activity assay

We followed the procedure of White & Karr (1978) with minor modifications. Our 80 μl incubation volume contained phosphate buffer (50 mM), cyclic AMP (3 mM), isobutylmethylxanthine (1.6 mM), phosphocreatine (18 mM), creatine phosphokinase (8.5 units), GTP (0.01 mM), $MgCl_2$ (2.3 mM), AMP (1 mM; approx. 300 nCi of [α-^{32}P]AMP), EGTA (0.12 mM) and bovine serum albumin (0.08 mg). The 40 min incubations (30 °C) were terminated by the addition of 0.15 ml of 1 M-$HClO_4$. Separation and counting of the labelled product followed the method of White & Karr (1978).

Protein assay

Protein was assayed by the method of Hartree (1972), with bovine serum albumin as standard. [The concentration as measured by this assay is consistently two or three times higher than that measured by the Bio-Rad protein assay, which is based on the method of Bradford (1976).]

Calcium buffer

Where a calcium buffer was used, the active ingredients were phosphate buffer (50 mM, pH 7.5 at 30 °C), EGTA (0.12 mM), *N*-hydroxyethylenediaminetriacetic acid (0.75 mM), ATP (1 mM) and $MgCl_2$ (2.3 mM). The stability constants were taken from Sillén & Martell (1964, 1971) and Martell & Smith (1974), and corrected, if necessary, to 30 °C.

Preparation of crude olfactory adenylate cyclase

Medium A was NaCl (0.9%)/EGTA (1 mM)/phosphate buffer (5 mM), pH 7.0; medium B was NaCl (0.9%)/phosphate buffer (5 mM), pH 7.0.

The following preparation was designed to minimize the use of organic chemicals, in case substances like (protective) mercaptans, organic buffers, proteolytic inhibitors and sucrose stimulate the olfactory receptors. Results suggest, however, that not all of these components interfere and that the preparation can be achieved by using rather more conventional buffer systems.

The ethmoturbinates were removed from a freshly killed rat and soaked in cold medium A to remove superficial blood and debris. The solution was changed three times at 3 min intervals with minimal agitation. The tissue was then sonicated in 10 vol. of medium B. Sonication was for 5 s in ice at the medium power setting of the MSE 100 W disintegrator using an exponentially tapered probe of 3 mm tip diameter. The maximum amount of material sonicated in any one tube was that derived from two rats.

The suspension was removed and the tissue rinsed with a further 10 vol. of medium B. The extracts were pooled and centrifuged at 1000 *g* for 30 min at 4 °C. The supernatant was withdrawn and re-centrifuged at 20000 *g* for 40 min at 4 °C. This pellet was resuspended

Abbreviation used: EOG, electro-olfactogram.

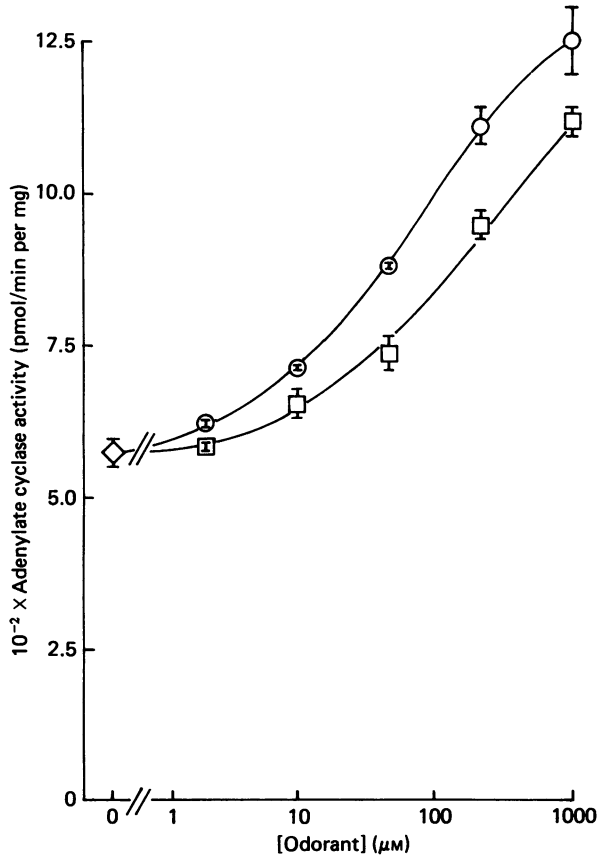

Fig. 1. Variation of adenylate cyclase activity with the concentration of added odorant

○, Carvone; □, acetophenone. The error bars indicate the S.E.M. of triplicate determinations on a single preparation.

in medium B, usually 2 vol. based on the original weight of tissue. Yields were typically 0.4–0.6 mg of protein per rat.

RESULTS

Adenylate cyclase activity (typically 500 pmol/min per mg of protein) was found in the olfactory preparations. Cyclic AMP production, under the conditions of the assay, was linear with time for up to 1 h.

Preparations showed enhanced cyclase activity when assayed in the presence of odorants (Fig. 1), and activity varied with the concentration of added odorant. The concentration range of the odorants shown in Fig. 1 is physiologically relevant, since the vapour pressures over the assayed solutions [calculated from the water/air partition coefficients (S. G. Shirley, E. H. Polak, D. A. Edwards, M. A. Wood & G. H. Dodd, unpublished work)] are about 0.003–3 Pa for carvone and 0.008–8 Pa for acetophenone.

The EOG is an indicator of the early electrical events in odour transduction (Ottoson, 1970). The variation of its amplitude with odorant concentration can be described by the equation:

$$\log A = m \times \log C + \text{constant} \quad (1)$$

(Ottoson, 1956; S. G. Shirley, E. H. Polak, D. A. Edwards, M. A. Wood & G. H. Dodd, unpublished work)

where C is the concentration of the odorant. For most odorants the coefficient m is in the range 0.3–0.5. The data in Fig. 1 also fit this equation. In this case A is the cyclase activity minus the activity with no added odorant. For carvone, m is 0.34 and the correlation coefficient (r) is 0.97; for acetophenone m is 0.43 and r is 0.99.

Ca^{2+}, at concentrations in and above the physiological range, inhibited the cyclase activity, in both the presence and the absence of odorant (Fig. 2).

Other odorants also stimulated the activity of olfactory preparations. At 1 mM, (+)-carvone produced nearly the same stimulation as the (−)-isomer. Isopentyl acetate and 1–8-cineole at 1 mM produced stimulations of 50 and 35% of the resting activity respectively. Hexanol and triethylamine at 3 mM gave stimulations of 61 and 55% respectively. (+)-Camphor stimulated the activity by 34% at half-saturated concentration.

We used glycerol as an example of a non-odorous organic material. This failed to stimulate the olfactory cyclase when it was applied at 30 mM.

Sucrose (a common component of membrane preparation media) at 270 mM in the assay mixture caused a doubling of the activity, and odorants added in the

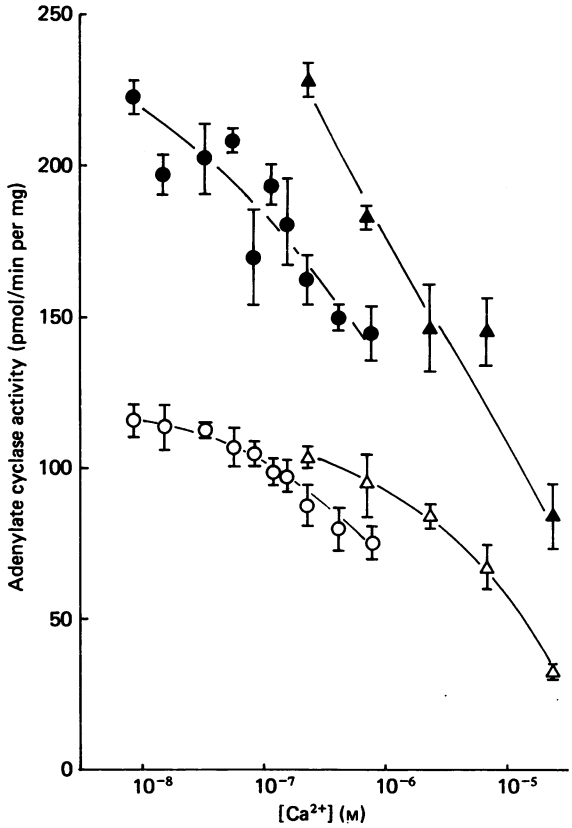

Fig. 2. Ca^{2+} inhibits both odour-stimulated and basal adenylate cyclase activity

The left-hand curves were measured in the presence of the calcium buffer described in the Materials and methods section. For the right-hand curves (different preparation) the 50 mM-phosphate buffer provided the calcium-buffering capacity. The error bars represent the S.E.M. of triplicate determinations. ○, △, No added odour; ▲, 1 mM-carvone; ●, 2 mM-acetophenone.

presence of sucrose caused no further stimulation. The above results are based on duplicate determinations whose variability was 4% (S.D.).

No stimulation of the cyclase was observed in the presence of either 1 mM-histamine or 1 mM-isoprenaline. NaF in the assay mixture at 10 mM caused a 2.5-fold increase in cyclase activity. Guanosine 5′-*O*-(3-thiotriphosphate) and guanosine, 5′-*O*-[$\beta\gamma$-imido]triphosphate, each at 0.1 mM, caused 3.8- and 2.8-fold increases respectively. Guanosine 5′-*O*-(2-thiodiphosphate) at 0.01 mM decreased the activity of the cyclase preparation to 20% of the initial level.

DISCUSSION

Pace *et al.* (1985) and Anholt (1986) have suggested tht olfaction in the frog is mediated by a cyclic AMP system. This may be true for the rat also; we have found an adenylate cyclase responding to physiologically relevant concentrations of odorants in a way which parallels the early electrical events of odour transduction. The effects of the guanine nucleotide analogues on the rat cyclase indicate the possible involvement of a G-protein, as has been demonstrated for the frog by Pace *et al.* (1985). An inhibition of the frog cyclase by Ca^{2+} has been shown by Anholt (1986).

Menevse *et al.* (1977) found that odorants did not stimulate the cyclase activity of olfactory membranes. These membranes were prepared in sucrose, which we have found to stimulate the cyclase and may have masked any response to odour.

Receptor heterogeneity (Polak, 1973) could explain the shape of the enzyme-activity-versus-odorant-concentration curve (Pace *et al.*, 1985). Some recent results (S. G. Shirley, E. H. Polak, D. A. Edwards, M. A. Wood & G. H. Dodd, unpublished work) on the variation of EOG amplitude with odorant concentration support this view.

In general, adenylate cyclase systems are compartmentalized within the cell (Earp & Steiner, 1978). The olfactory transduction mechanism is believed to reside in the cilia and terminal swelling of the olfactory primary cells (Getchell *et al.*, 1984). The high levels of cyclase activity which we have observed would, if confined to this region, be sufficient to explain the speed of response of the olfactory system. Our data, combined with the ultrastructural data of Menco (1980), would imply cyclase activities of about 10^{-18} mol/s per cell (minimum). The combined volume of terminal swelling and cilia is 5×10^{-15} l/cell (Menco, 1977). With a doubling of activity on stimulation, the local cyclic AMP concentration should rise with an initial rate of some 200 μM/s. Changes in cyclic AMP concentrations of the order of 1 μM are of physiological significance. [Terasaki & Brooker (1977) estimated that half-maximal binding of cyclic AMP in rat heart occurred at free concentrations of about 1 μM.] The response time of the system should therefore be in the millisecond range.

The olfactory adenylate cyclase should be a powerful tool for the study of the olfactory receptors *in vitro*, providing a monitor for the solubilization and purification of the receptors.

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