

These results and those obtained by Matsuzawa & Nirenberg (1975) suggest that occupancy of the low-affinity agonist-binding site of the muscarinic acetylcholine receptor leads to stimulation of a guanylate cyclase in NIE 115 neuroblastoma cells. There is evidence, however, (P. G. Strange, unpublished work; Michell, 1975) that cyclase activation is not the first event after receptor occupancy and that a receptor-mediated Ca^{2+} influx and/or phospholipid turnover may precede this event.

P. G. S. thanks the M.R.C. for the award of a Training Fellowship. We thank Dr. Kay Fields for helpful advice and for the gift of NIE 115 neuroblastoma.

- Amano, T., Richelson, E. & Nirenberg, M. (1972) *Proc. Natl. Acad. Sci. U.S.A.* **69**, 258–263
 Birdsall, N. J. M. & Hulme, E. C. (1976) *J. Neurochem.* **27**, 7–16
 Bowman, W. C., Rand, M. J. & West, G. B. (1971) *Textbook of Pharmacology*, Blackwell, London
 Burgen, A. S. V. & Spero, L. (1968) *Br. J. Pharmacol.* **34**, 99–115
 Matsuzawa, H. & Nirenberg, M. (1975) *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3472–3476
 Michell, R. H. (1975) *Biochim. Biophys. Acta* **415**, 81–147
 Snyder, S. H., Chang, K. J., Kuhar, M. J. & Yamamura, H. I. (1975) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **34**, 1915–1921
 Terenius, L. (1974) *Acta Pharmacol. Toxicol.* **34**, 88–91
 Yamamura, H. I. & Snyder, S. H. (1974) *Proc. Natl. Acad. Sci. U.S.A.* **71**, 1725–1729

A Chemical-Modification Approach to the Olfactory Code

ADNAN MENEVSE,* GEORGE H. DODD,* T. MICHAEL POYNDR†
 and DAVID SQUIRREL*

**Department of Molecular Sciences, University of Warwick, Coventry CV4 7AL, U.K.,*
 and †*Department of Biology, Guy's Hospital Medical School, London SE1 9RT, U.K.*

There are two outstanding biochemical problems involved in the olfactory process. First, there is the nature of the transduction mechanism whereby the interaction of an odorant with the primary olfactory neurons initiates a receptor potential. A number of mechanisms have been proposed for the transduction step (Poynder, 1974). We have provided direct evidence for the involvement of an adenylate cyclase in this process (Menevse *et al.*, 1976). Our evidence suggests that odorants behave as regulatory ligands of this enzyme (Dodd, 1976). The second biochemical problem for olfaction is the nature of the coding mechanism for odour quality, which operates at the level of the primary neurons. The evidence on the response specificity of individual olfactory neurons suggests that these cells are generalist cells which respond in a differential manner to a wide range of odorants (Gesteland, 1976). This evidence, together with the occurrence of a number of specific anosmias (Amoore, 1974), suggests that there are at least 30 types of olfactory receptor proteins, which are distributed in differing relative concentrations among the primary neurons. These receptor proteins can then be thought of as regulatory subunits of the plasma-membrane adenylate cyclase. On the basis of this model of the olfactory mechanisms, the problem of the olfactory code in the primary neurons can be reduced to the identification of the number of independent receptor proteins, together with the estimation of the possible types of interactions between them.

Chemical modification of ligand-binding sites is a classical approach to this kind of problem, and it has been used on both insect olfaction (Frazier & Hertz, 1975) and vertebrate olfaction (Frazier & Hertz, 1975; Getchell & Gesteland, 1972). We describe here some results from three complementary methods of chemical modification which demonstrate that it is feasible to undertake a study of the olfactory code by these methods.

(1) *Vapour-phase modification by using group-specific reagents*

In this type of experiment we used volatile reagents with a vapour pressure sufficient to produce a high concentration of reagent in the vapour phase. The advantage of such reagents is that the molecules have access to the olfactory receptors in the same manner

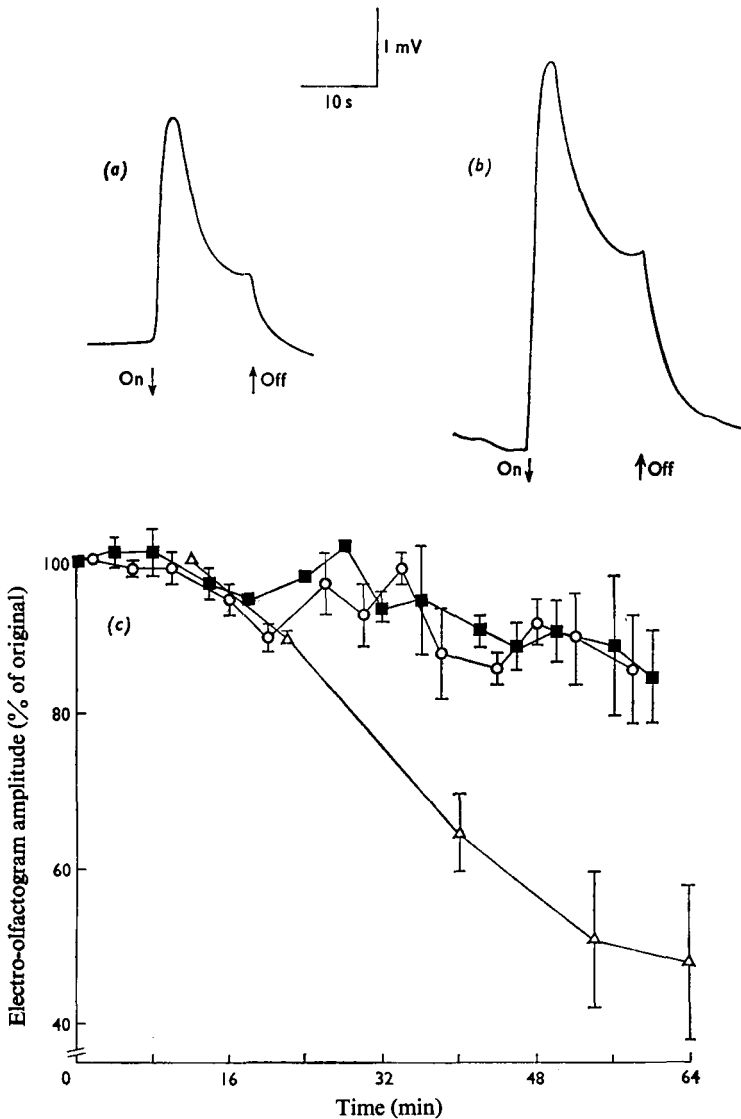


Fig. 1. Specific labelling of receptors for the odorant 4-chloro-7-nitrobenzofurazan

(a) Typical electro-olfactogram for this odorant; (b) typical electro-olfactogram for *n*-amyl acetate. The points in (c) are means \pm S.E.M. of three experiments. ■, 1,8-Cineole; ○, *n*-amyl acetate; △, 4-chloro-7-nitrobenzofurazan. The vapour pulses were of 10 s duration, with an interval of 2 min between each. The general experimental methods were as described by Getchell & Gesteland (1972).

as the odorants, thus there is no disturbance of the mucus layer which lies directly over the olfactory tissue. Among the compounds used were 4-chloro-7-nitrobenzofurazan, *N*-ethylmaleimide, 1-fluoro-2,4-dinitrobenzene and benzyl chloride. All of these compounds behaved as odorants and produced receptor potentials which were recorded as the electro-olfactogram by using standard methods (Getchell & Gesteland, 1972;

Poynder, 1974, pp. 241–249). The electro-olfactogram response of 4-chloro-7-nitrobenzofurazan in response to stimulation with a square-wave vapour pulse is shown in Fig. 1, and it exhibits a normal peak and plateau in comparison with *n*-amyl acetate. After repeated stimulation of the tissue with pulses of these reagents, the electro-olfactogram responses of these reactive chemicals decreased irreversibly, relative to those of non-reactive odorants, indicating that they were selectively labelling specific receptor sites (Fig. 1). Irreversible inhibition of the electro-olfactograms was also brought about when solutions of these reagents were added to the tissue. However, the selectivity of the labelling found in the vapour-phase experiments was not demonstrable when the reagents were used in the liquid phase.

(2) Vapour-phase labelling by using photoaffinity odorants

A disadvantage of the group-specific reagents is that they are irritant chemicals and they probably stimulate trigeminal receptors as well as olfactory receptors. Aromatic azido compounds, however, are chemically unreactive, but decompose to reactive short-lived intermediates on u.v. irradiation. Since the physicochemical properties of the azido group substituted in an aromatic nucleus are similar to those of an aldehyde group in an aromatic nucleus we can expect that many aromatic azido compounds will be pleasant-smelling odorants. Phenylazide and 1-azidonaphthalene, for example, are both pleasant-smelling liquids and they behave as normal odorants for the frog, giving typical electro-olfactograms of the kind shown in Fig. 1. After illumination of the frog olfactory

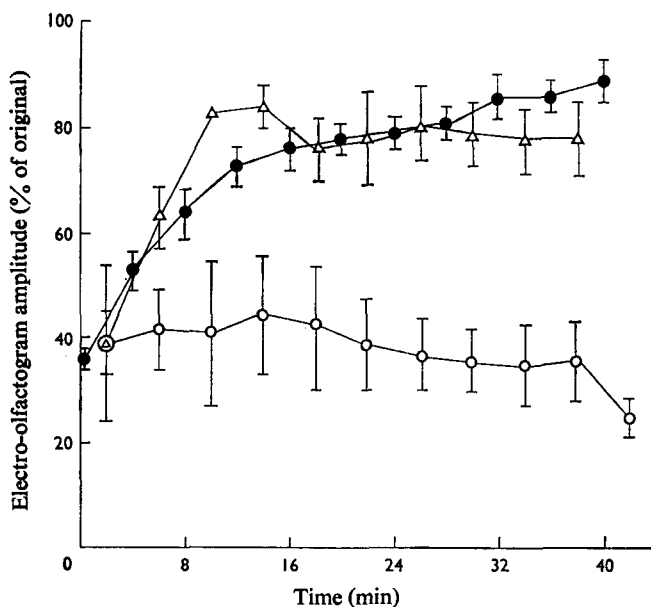


Fig. 2. Specific labelling of receptor sites by mersalyl and protection by an odorant

This experiment was performed essentially along the lines of the experiments of Getchell & Gesteland (1972). The tissue was washed with Ringer's solution for 10 min, then the reagent was applied for 10 min, after which the tissue was washed with Ringer's solution for 10 min. The electro-olfactograms were then recorded as a fraction of time by using a 10 s vapour pulse, with an interval of 2 min between pulses. *n*-Amyl acetate was present in all of the solutions applied to the tissue. The points are shown as the mean \pm S.E.M. of three experiments. ●, *n*-Amyl acetate; Δ , *n*-butyl acetate; ○, β -ionone.

mucosa with the light from a xenon lamp (by using a glass filter to cut off light below 310nm) during constant stimulation with 1-azidonaphthalene vapour, there was a specific partial inactivation of the receptors for this odorant, after sufficient time had been allowed for the tissue to recover from adaptation effects. In control experiments under the same irradiation conditions, the electro-olfactogram responses to the odorants naphthalene and ethyl *n*-butyrate did not change by more than 10% of the original value. The extent of inactivation of the electro-olfactogram response to 1-azidonaphthalene varied between 60 and 100% of the original value, depending on the experimental conditions. The results of this experiment suggest that photoaffinity odorants are promising and novel agents for the specific labelling of olfactory receptors.

(3) Modification in the liquid phase by using a non-penetrant thiol reagent and specific protection on the sites by odorants

The thiol reagent *N*-ethylmaleimide has been shown to abolish irreversibly the electro-olfactogram response to the fruity odorant ethyl *n*-butyrate when a solution of the reagent is applied to the olfactory epithelium (Getchell & Gesteland, 1972). The labelled sites could be protected if a high concentration of the odorant (10 mM) was included in the solution of the reagent. Since *N*-ethylmaleimide can penetrate membranes, it is possible that the effects found with this agent were not due to specific modification of a receptor on the exterior of the neuronal plasma membrane. We have carried out similar experiments using the non-penetrant thiol agent mersalyl {*o*-[(3-hydroxymercuri-2-methoxypropyl)-carbamoyl]phenoxyacetic acid sodium salt}.

The results of a typical experiment are shown (Fig. 2) and demonstrate specific protection of receptor sites for the odorant amyl acetate, by both the odorant and the congener *n*-butyl acetate, together with a lack of protection by the unrelated odorant β -ionone.

We thank the M.R.C. for financial support and the Royal Society for an equipment grant. A. M. was supported by a grant from the Turkish Government.

Amoore, J. E. (1974) *Ann. N.Y. Acad. Sci.* **237**, 137-143

Dodd, G. H. (1976) in *Structure-Activity Relationships in Chemoreception* (Benz, G., ed.), pp. 55-61, Information Retrieval, London

Frazier, J. L. & Hertz, J. R. (1975) *Chem. Senses and Flavor* **1**, 271-281

Gesteland, R. C. (1976) in *Structure-Activity Relationships in Chemoreception* (Benz, G., ed.), pp. 161-168, Information Retrieval Ltd., London

Getchell, M. L. & Gesteland, R. C. (1972) *Proc. Natl. Acad. Sci. U.S.A.* **69**, 1494-1498

Menevse, A., Squirrell, D., Dodd, G. H. & Poynder, T. M. (1976) *Abstr. Congr. Eur. Chemoreception Research Organization*, 2nd 46

Poynder, T. M. (ed.) (1974) *Transduction Mechanisms in Chemoreception*, Information Retrieval Ltd., London

Isolation of Oligodendrocytes and Other Cell Lines from Whole Rat Brain Tissue

SE-WAN CHAO and MARTIN G. RUMSBY

Department of Biology, University of York, Heslington, York YO1 5DD, U.K.

Up to now it has not been possible to isolate the three principal cell lines of nerve tissue (neurons, astrocytes and oligodendrocytes) from whole rat brain tissue for subsequent analysis and investigation. When whole rat brain is used as the starting material, only neurons and astrocytes are recovered in significant yield at the end of the preparation (Rose, 1965; Norton & Poduslo, 1970; Sellinger *et al.*, 1971). Oligodendrocytes have been separated from rat brain tissue, but only if the white matter is separated free from grey matter first, a very tedious operation (Fewster *et al.*, 1967). In our work we need to be able to isolate all three cell lines from the same rat brain for metabolic studies on cell-