carried out at the molecular level. The isolation of the terminal swelling of olfactory neurones (Koch & Norring, 1969) has now made it feasible to investigate the molecular basis of the receptor mechanisms.

In a typical preparation starting with about 20 large rabbits the yield of purified terminal swellings was about 200 μ g of protein. Neither high-resolution n.m.r. nor electron spin resonance with nitroxide probes was sufficiently sensitive to detect odorant binding at the membrane concentration available.

Fluorescence probe methods were sufficiently sensitive to detect odorant binding to the olfactory terminal swellings. The fluorescent probe 1-anilinonaphthalene-8-sulphonate interacts with the membranes. The binding of this probe to the terminal swellings resembled the binding to other membranes, e.g. erythrocyte membranes (Freedman & Radda, 1969), and differed from most proteins, e.g. glutamate dehydrogenase (Dodd & Radda, 1969), in that an indefinite number of probe sites was found. Aromatic odorants formed a ground-state complex with 1-anilinonaphthalene-8-sulphonate, but most aliphatic and alicyclic odorants did not alter the fluorescence of the probe. Butyric acid was chosen as the most suitable odorant for these experiments. This odorant reproducibly induces, in the 10^{-4} concentration range, characteristic changes in the terminal swellings that are reflected in a 1.2-fold enhancement of the fluorescence of bound 1-anilinonaphthalene-8-sulphonate. Other odorants give similar changes. Similar changes are seen when a sonicated egg-yolk lecithin suspension containing 1-anilinonaphthalene-8-sulphonate is titrated with butyric acid and other odorants.

These observations have been interpreted as evidence of odorant binding to a phospholipid region of the olfactory sensory membranes rather than to any receptor proteins. The results suggest that localized structural changes in membrane lipids give rise to the receptor potential. This interpretation is compatible with our studies on a model system for olfactory transduction (Cherry, Dodd & Chapman, 1970).

Cherry, R. J., Dodd, G. H. & Chapman, D. (1970). *Biochim. biophys. Acta*, **211**, 409.

Dodd, G. H. & Radda, G. K. (1969). *Biochem. J.* **114**, 409.

Freedman, R. B. & Radda, G. K. (1969). *FEBS Lett.* **3**, 150.

Gestland, R. C., Lettvin, J. Y. & Pitts, W. H. (1965). *J. Physiol., Lond.*, **181**, 525.

Koch, R. B. & Norring, N. L. (1969). *J. Neurochem.* **16**, 145.

Trans-synaptic Induction of Dopamine (3,4-Dihydroxyphenethylamine) β -Hydroxylase in Adrenergic Tissues of the Rat

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A sensitive enzymatic assay for the enzyme dopamine (3,4-dihydroxyphenethylamine) β -hydroxylase was used to study changes in enzyme activity that occur with pharmacologically induced alterations in the activity in the sympathetic nervous system. The assay is based on the enzymic conversion of the β -hydroxylated product of the reaction into its *N*-methyl derivative in the presence of *S*-adenosyl[Me- 14 C]methionine (Molinoff, Weinshilboum & Axelrod, 1971). Endogenous inhibitors of dopamine β -hydroxylase were inactivated with copper sulphate.

The activities of dopamine β -hydroxylase (Molinoff, Brimijoin, Weinshilboum & Axelrod, 1970) and of tyrosine hydroxylase (Mueller, Thoenen & Axelrod, 1969) were increased in adrenergic tissues of the rat by agents, including reserpine, that are believed to cause a reflex increase in sympathetic tone. After reserpine treatment dopamine β -hydroxylase activity increased within 24h in sympathetic ganglia. In the heart and, as previously reported, in the adrenal gland (Viveros, Arqueros, Connett & Kirshner, 1969) the increase in activity was preceded by a fall that occurred within a few hours of the injection. The activities of several enzymes not involved in catecholamine biosynthesis were not affected by reserpine.

When efferent-nerve impulse activity was blocked by either surgical decentralization or by ganglion-blocking agents, the effect of reserpine was abolished. Monoamine oxidase inhibitors, bretylium, which blocks the neurally induced release of noradrenaline, and catecholamines themselves all interfere with the ability of reserpine to release catecholamines and all blocked the induction of dopamine β -hydroxylase in ganglia. The administration of the catecholamines dopa (3,4-dihydroxyphenylalanine) and dopamine also caused a marked decrease in cardiac dopamine β -hydroxylase activity.

The observed increase in dopamine β -hydroxylase activity appears to represent the synthesis of new enzyme and not the activation of pre-existing enzyme. Thus the K_m for phenethylamine, 6×10^{-4} M, and the electrophoretic mobility of dop-

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amine β -hydroxylase on polyacrylamide gels were the same for the enzyme from reserpine-treated rats as for that from control animals (S. Ross, R. Weinshilboum, P. B. Molinoff, E. Vesell & J. Axelrod, unpublished work); and the increase in dopamine β -hydroxylase activity was blocked by the protein-synthesis inhibitor cycloheximide. After administration of cycloheximide the dopamine β -hydroxylase activity in sympathetic ganglia decreased with a half-life of about 15 h both in control animals and in chronically reserpinated animals.

It is concluded that long-term changes in dopamine β -hydroxylase activity in the sympathetic nervous system result in changes in the amount of activity in the neuron. These changes are trans-synaptic effects of nerve impulses and require protein synthesis.

- Molinoff, P. B., Brimijoin, S., Weinshilboum, R. & Axelrod, J. (1970). *Proc. natn. Acad. Sci. U.S.A.* **66**, 453.
Molinoff, P. B., Weinshilboum, R. & Axelrod, J. (1971). *J. Pharmac. exp. Ther.* (in the Press).
Mueller, R. A., Thoenen, H. & Axelrod, J. (1969). *J. Pharmac. exp. Ther.* **169**, 74.
Viveros, O. H., Arqueros, L., Connett, R. J. & Kirshner, N. (1969). *Molec. Pharmac.* **5**, 69.

peritoneal route has been found most convenient. Similar results have been reported by Miller & Emmens (1967).

A dose-response curve of the incorporation of precursors into RNA shows a maximum at $0.3 \mu\text{g}$ of oestradiol- 17β /rat, but even at $0.01 \mu\text{g}$ /rat there is a 4-fold stimulation.

Variation in rat weight also influences the effect of oestradiol on incorporation of precursors into RNA. Depending partly on litter size, the weights of 18–21-day-old rats varied between 20 and 40 g. When selected on the basis of weight and age, 20–25 g rats gave the greatest response to oestradiol, peaking at 12 times the control value 4 h after hormone administration; 25–30 g rats, which make up the largest proportion, peaked at 10 times the control, and 30–35 g and 35–40 g rats gave 7.5-fold and less than 6-fold increases respectively.

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- Billing, R. J., Barbiroli, B. & Smellie, R. M. S. (1969). *Biochem. J.* **112**, 563.
Gorski, J. & Nicolette, J. A. (1963). *Archs Biochem. Biophys.* **103**, 418.
Miller, B. G. & Emmens, C. W. (1967). *J. Endocr.* **39**, 473.

Oestradiol- 17β Stimulation of Ribonucleic Acid Synthesis in Immature Rat Uterus: the Effects of Injection Route and Rat Weight

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The synthesis of RNA in immature rat uterus is stimulated by oestradiol- 17β (Gorski & Nicolette, 1963; Billing, Barbiroli & Smellie, 1969). When measured in terms of incorporation of tritiated ribonucleosides into acid-insoluble material the route of administration of the labelled precursor exerts a profound effect on the response to oestradiol. In our hands intraperitoneal injection of the precursor, the route chosen by most workers, gives a low and very variable response to the hormone. Increases of 3–4-fold and 2–2.5-fold were observed in uptake into the acid-insoluble and acid-soluble fractions respectively, but there was considerable variation between duplicate animals. Intravenous injection of the labelled precursor consistently produced 8–11-fold stimulation in acid-insoluble radioactivity and 2–2.5-fold increases in acid-soluble radioactivity in the hormone-treated animals. Subcutaneous injection produces an intermediate response. The mode of hormone administration has little influence and the intra-

Separation of Ribonucleic Acid from Immature Rat Uterus on Polyacrylamide Gels and the Effect of Oestradiol- 17β

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Tritiated ribonucleosides were administered intravenously to 18–21-day-old rats weighing 25–30 g. RNA was prepared from the uteri by the method of Joel & Haggerman (1969) and shown to be undegraded and free from DNA. It was fractionated on acrylamide–ethylene diacrylate gels as described by Loening (1969) and Weinberg, Loening, Willems & Penman (1967).

The earliest response to oestradiol was found in an RNA species remaining at the origin of the gel from animals that were treated with hormone 30 min–1 h before death. This RNA makes up a small proportion of the total RNA as judged by extinction measurements, but even in controls it represents a high proportion of the total radioactivity in RNA. Present evidence indicates that it is not methylated and, though it is sensitive to ribonuclease, it is insensitive to Pronase. By 1 h after oestradiol treatment short pulses of labelled precursors show that ribosomal precursor RNA species are becoming more heavily labelled, and by