Labelling of Cholinergic Receptors in Subcellular Fractions from Rat Cerebral Cortex

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Irreversible antagonists, specific to a particular receptor type and radioactively labelled, have proved a powerful tool in studies towards the isolation of pharmacological receptors, and the principle of affinity labelling has been increasingly employed for the production of suitable compounds (Changeux *et al.*, 1967; Karlin & Winnick, 1968; Kiefer *et al.*, 1970; Bartels *et al.*, 1971; Karlin *et al.*, 1971). The presence of chemically reactive groups in these antagonists demands that, when used for the purposes of receptor labelling, sufficiently low concentrations can be employed to minimize reaction with the great excess of non-receptor material. Benzilylcholine mustard (Gill & Rang, 1966; Rang, 1967; Fewtrell & Rang, 1971) fulfils this requirement for the muscarinic acetylcholine receptor of the guinea-pig ileum. In this communication we describe the use of a ^3H -labelled homologue of benzilylcholine mustard, *N*-2'-chloroethyl-*N*-[2'',3''- $^3\text{H}_2$]propyl-2-aminoethyl benzilate (specific radioactivity 1.4 Ci/mmol), to label receptors with muscarinic character in subcellular fractions from rat cerebral cortex.

The crude mitochondrial pellet obtained from rat cerebral cortex homogenized in iso-osmotic sucrose was separated into three fractions, designated myelin, synaptosomal and mitochondrial, on a discontinuous sucrose-density-gradient essentially as described by Whittaker (1959). Atropine-sensitive binding of *N*-2'-chloroethyl-*N*-[2'',3''- $^3\text{H}_2$]propyl-2-aminoethyl benzilate was found mainly in the myelin and synaptosomal fractions. Further investigation of the synaptosomal fraction showed that the atropine-sensitive binding was saturatable, whereas the atropine-insensitive uptake continued to increase linearly with time. Assuming a 1:1 reaction of *N*-2'-chloroethyl-*N*-[2'',3''- $^3\text{H}_2$]propyl-2-aminoethyl benzilate with the receptor, the receptor concentration in this fraction is approx. 400 pmol/g of protein. Half-maximal inhibition of the atropine-sensitive binding occurred with approx. 5 nM-atropine. Isopropamide, a potent muscarinic antagonist, inhibited the binding of *N*-2'-chloroethyl-*N*-[2'',3''- $^3\text{H}_2$]propyl-2-aminoethyl benzilate to the same extent as atropine, but (+)-tubocurarine, decamethonium, eserine and neostigmine, all used at concentrations of 100 nM, were without effect.

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Glyceride Lipases in Brain and their Stimulation by Noradrenaline and 5-Hydroxytryptamine

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Triglyceride lipase, diglyceride lipase and monoglyceride lipase were determined in homogenates and nerve endings of combined cerebral cortex and cerebellum of guinea pig. Nerve endings (synaptosomes) were isolated (Eichberg *et al.*, 1964) and osmotically ruptured by suspension in water (2 ml/g of tissue; Whittaker *et al.*, 1964). Tissue was incubated with emulsions of tri[1- ^{14}C]palmitoylglycerol, 1,2-di[1- ^{14}C]palmitoylglycerol or 2-[1- ^{14}C]palmitoylglycerol in 1 ml of 50 mM-tris buffer, pH 7.4. Hydrolysis was determined by measurement of the [1- ^{14}C]palmitate released.

The rates of release of palmitate from tripalmitin, dipalmitin and monopalmitin were in the ranges 4–21, 274–500 and 2100–5900 $\mu\text{mol/h}$ per g of protein respectively. Protein concentration was 0.03–0.05 mg/ml for measurement of monoglyceride lipase. With osmotically ruptured synaptosomes the rate of hydrolysis of 1,2-di[1- ^{14}C]palmitoylglycerol mixed with non-radioactive 1,3-dipalmitoylglycerol was the same as when mixed with non-radioactive 1,2-dipalmitoylglycerol–1,3-dipalmitoylglycerol mixture (3:7, w/w), indicating that the system did not distinguish between the two diglycerides. There was no difference between the rates of hydrolysis of 2-palmitoylglycerol and 1-palmitoylglycerol.

The three glyceride lipases were found in all particulate subcellular fractions. Triglyceride lipase activity was low in the supernatant and microsomal fractions. Diglyceride lipase was concentrated to a small extent in the mitochondrial fraction. The pH optima of triglyceride lipase, diglyceride lipase and monoglyceride lipase were approx. 7.4, 7.4 and 8 respectively in synaptosomes, and approx. 7.4, 7–8 and 8 respectively in homogenates. Triglyceride