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.

C. R. H. holds a Medical Research Council Scholarship for Training in Research Methods.

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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

lipase and diglyceride lipase of synaptosomes had apparent K_m values approx. 1.5 and 0.5mM respectively.

Triglyceride lipase activity in osmotically ruptured synaptosomes was stimulated 49% ($P < 0.001$) by 1.0mM-noradrenaline and 24% ($P < 0.001$) by 1.0mM-5-hydroxytryptamine. Noradrenaline (1.0mM) was without effect on the triglyceride of microsomal material and depressed slightly (11%; $P < 0.02$) the triglyceride lipase activity of mitochondria. Diglyceride lipase activity was stimulated 35% ($P < 0.001$) by noradrenaline (1.0mM) and 21% ($P < 0.001$) by 5-hydroxytryptamine (1.0mM). Monoglyceride lipase activity was unaffected by either noradrenaline or 5-hydroxytryptamine. Since nerve endings of brain contain noradrenaline and 5-hydroxytryptamine (Michaelson & Whittaker, 1963; Michaelson *et al.*, 1963; Zieher & De Robertis, 1963; Levi & Maynert, 1964) bound to synaptic vesicles (Michaelson *et al.*, 1963; Maynert *et al.*, 1964), similar changes may take place with endogenous glycerides *in vivo* during synaptic transmission.

O. S. V. is grateful to the Medical Research Council for a Research Scholarship.

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The Synthesis of Mercapturic Acids from Allyl Compounds in the Rat

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In previous investigations (Clapp *et al.*, 1969; Kaye & Young, 1970) the presence of mercapturic acids in the urine of rats dosed with a number of allyl compounds was demonstrated. It appeared that allyl halides and esters of allyl alcohol derived from strong acids give rise to the excretion of allylmercapturic acid and 3-hydroxypropylmercapturic acid, i.e. *N*-acetyl-*S*-allyl-L-cysteine and *N*-acetyl-*S*-(3-hydroxypropyl)-L-cysteine, in the urine of the dosed animals. On the other hand, allyl alcohol and its esters with weak acids were found to give rise to

the formation of 3-hydroxypropylmercapturic acid, but not to allylmercapturic acid.

In the present work further support for this pattern in the metabolism of allyl compounds has been obtained. After the administration of allyl formate or allyl propionate to rats no allylmercapturic acid was detected in the urine, but it was possible to isolate 3-hydroxypropylmercapturic acid, as its dicyclohexylammonium salt, in amounts representing 6.8 and 9.1% respectively of the administered ester. Further, diallyl phthalate and allyl nitrite were found to give rise to the formation of 3-hydroxypropylmercapturic acid, but not to allylmercapturic acid. Administration of an ester of allyl alcohol derived from a strong acid, however, namely allyl nitrate, led to the isolation of both allylmercapturic acid and 3-hydroxypropylmercapturic acid, the latter as its dicyclohexylammonium salt, from the urine of the dosed rats in amounts corresponding to 5.9 and 2.6% respectively of the injected ester.

Two other allyl compounds, allylamine and allyl cyanide, were shown to lead to the excretion of 3-hydroxypropylmercapturic acid, but not allylmercapturic acid, in the urine of the dosed rats.

Gray & Barnsley (1971) have suggested that the metabolic formation of 3-hydroxy-1-methylpropylmercapturic acid from crotyl alcohol occurs with the formation of crotonaldehyde as an intermediate. In the present work, 3-hydroxypropylmercapturic acid, as its dicyclohexylammonium salt, was isolated in 10.5% yield from the urine of rats dosed with acrolein, and there are therefore grounds for believing that this aldehyde may be an intermediate in the metabolic conversion of allyl alcohol into a mercapturic acid. No evidence was found for the excretion of allylmercapturic acid by rats dosed with acrolein.

The metabolic pathway for the formation of mercapturic acids from allyl compounds was discussed.

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A New Micro Method for the Determination of Acid Glycosaminoglycans in Urine

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Procedures currently available for the determination of total urinary glycosaminoglycans tend to be lengthy and complicated. Methods include precipitation with quaternary ammonium salts, which is the most widely used (Di Ferrante & Rich, 1956; Teller