Radiochemical assay of catecholamines in blood plasma

M.A. BARRAND, B.A. CALLINGHAM, J.P. FRY, M.J. MITCHINSON & D.F. SHARMAN

Departments of Pathology and Pharmacology, University of Cambridge and the Agricultural Research Council Institute of Animal Physiology, Babraham, Cambridge.

At present, the most promising approach to the assay of the very small quantities of the three catecholamines, known to be present in normal blood plasma, is the use of a suitable enzyme that allows the formation of identifiable radioactively labelled products. Passon & Peuler (1973) used the method of Engelman, Portnoy & Lovenberg (1968), in which a preparation of the enzyme catechol-O-methyl transferase enables the introduction of a radioactively labelled methyl group into the catecholamine molecule, and were able to carry out the reaction directly in small volumes of blood plasma. Fry, House & Sharman (1974) estimated the content of the catecholamines in the salivary gland of the cockroach after separation of such radioactive compounds, as their acetylated derivatives, by paper chromatography. This method of paper chromatographic separation has been preferred in the assay to be demonstrated since it allows the complete separation of acetylated, radioactive metanephrine, normetanephrine and methoxytyramine to be made with little difficulty. The acetylation of the methoxy-amines increases their stability so that there is little or no loss during chromatographic separation. We feel that there are fewer hazards in handling the paper chromatograms than with other methods of separation since the radioactive regions are simply cut out and eluted in the scintillation vial.

The demonstration will show the method used for the simultaneous estimation of noradrenaline, adrenaline and dopamine in the same sample of blood plasma and in small pieces of tissue. The reproduceability and sensitivity of the method will be illustrated and some results which have been obtained in studying widely different problems concerning catecholamines will be presented to demonstrate the suitability of the method for the screening of clinical material.

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A method for the micro-injection into mammalian muscle fibres of procion brilliant red H3BN

J.B. HARRIS & R.R. RIBCHESTER

Muscular Dystrophy Group Research Laboratories, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne, NE4 6BE, England

A variety of techniques for the preparation and use of micropipettes filled with Procion dyes have been described (see Kater & Nicholson, 1973). The present method is simple and deviates little from the method used in our laboratories for the preparation of conventional recording microelectrodes.

Procion Brilliant Red H3BN, when obtained from source (I.C.I. Limited, Dyestuffs Division), is contaminated with a water insoluble de-dusting agent (Stead, 1973). This agent was first removed by rinsing with acetone. A solution of the dye (4% w/v) was then prepared by dissolving this partially purified product in distilled de-ionized water and filtering the solution through a 0.45 μm millipore filter. Micropipettes were pulled from 2 mm bore pyrex glass tubing. They were filled by boiling under reduced pressure with industrial methylated spirit or absolute ethanol, transferred to distilled water for 5 minutes, and then immersed overnight in a dye solution. Pipettes thus filled with Procion Brilliant Red had tip resistances of 20-30 MΩ and tip potentials less than 10 mV. Conventional microelectrodes filled with 3M KCl prepared by a similar procedure had tip resistances of 5-15 MΩ and tip potentials less than 10 mV. Dye-filled pipettes prepared in this manner and stored in Procion Brilliant Red