Central uptake and cardiovascular effects of δ-aminolaevulinic acid

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A characteristic feature of acute intermittent porphyria is the increased biosynthesis of the porphyrins and their precursors δ-amino-laevulinic acid (ALA) and porphobilinogen. Although the purified porphyrins and porphobilinogen are pharmacologically inactive, ALA has recently been shown to inhibit both membrane sodium transport (Isaacson, Douglas & Eales, 1971) and brain ATPase activity (Becker, Viljoen & Kramers, 1971) *in vitro*. It is possible that some such action of ALA could be a contributory factor in the pathogenesis of acute porphyria.

There have been conflicting reports on the ability of ALA to pass the blood-brain barrier in the rat. It was found that at plasma concentrations known to occur in acute porphyria, (1–24 μg/ml), ALA could pass the blood-brain barrier. ALA was found also to cause a short lasting hypotensive response in the anaesthetized and pithed rats. This response could be elicited by a dose of 1 mg/kg but usually a dose of 2–4 mg/kg was employed. The response to these larger doses was normally an immediate fall of 10–30 mmHg gradually returning to normal over 30 s to 5 min. It is unlikely that this action is centrally mediated as the hypotensive response was still evident in the pithed rat when the blood pressure was raised by vasopressin. It is more likely that ALA causes vasodilatation either directly, or by the release of histamine. The ALA response is qualitatively similar to that of histamine, and shows many of the characteristics of a typical 'histamine releaser' response.

Further elucidation of the pharmacological actions of ALA, particularly in the C.N.S., may considerably increase our understanding of the mechanisms and course of acute porphyria.

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The relationship between ethyl oleate/Krebs solution partition coefficient and depression of myocardial contractile force in a number of pharmacologically active compounds (T)

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The prediction of drug activity (T)

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DEMONSTRATIONS

Use of dansyl chloride to detect amino acids and 5-hydroxytryptamine in small quantities of tissue

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It is generally accepted that certain amino acids and 5-hydroxytryptamine (5-HT) are involved in synaptic transmission in the invertebrate and vertebrate central nervous system. A sensitive microbiochemical method has therefore been developed by Neuhoff

and co-workers (Osborne, Briel & Neuhoff, 1971; Briel, Neuhoff & Maier, 1972; Neuhoff, 1973) which has enabled amino acids, 5-HT and related substances to be estimated in characterized invertebrate neurones and milligram quantities of nervous tissue (Osborne 1973, Osborne & Neuhoff, 1973).

This method involves the extraction of amines and free amino acids from tissue. Dansyl chloride is then reacted with the extracted substance at alkaline pH to form highly fluorescent dansyl derivatives. The derivatives are then separated by two dimensional chromatography on 3×3 cm polyamide plates.

The sensitivity of this method has been considerably increased by the use of ^{14}C-dansyl chloride (98 mCi/mM, Schwarz/Mann, Orangeburg, New York). This enables autoradiograms of the plates to be prepared thereby often allowing the detection of substances which are present in quantities barely visible in UV light. It is also possible to mark the perimeters of the fluorescent spots, remove them from the plate using a microknife and determine the radio-activity of the dansyl derivatives in a liquid scintillation counter. This method detects as little as 1 picomole of an amino acid or 5-HT.

A practical demonstration of this method will be given. Detailed results which have been obtained from the use of the dansyl method to analyse characterized invertebrate neurones and defined areas of vertebrate brain tissue, will be presented.

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The use of model droplets in monoamine histochemistry and some problems of colour photomicrography

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The relationship between the concentration of noradrenaline (NA) and that of 5-hydroxytryptamine (5-HT) and their fluorescence intensity and colour of fluorescence was investigated with the help of model experiments using droplets, the preparation and fluorescence characteristics of which are described.

5 μl droplets containing noradrenaline bitartrate (0·1 to 44 mg/ml) and 5-hydroxytryptamine creatinine sulphate (0·1 to 8·0 mg/ml) in 5 per cent albumin solution were dried on microscope slides at room temperature. In the case of 5-HT in order to increase the amount of this monoamine in the droplets, 5-hydroxytryptamine creatinine sulphate (8 mg/ml) was dried repeatedly on the same areas. The slides were treated with formaldehyde according to the method described by Falck & Owman (1965). Fluorescence was observed and fluorescence spectra were recorded with Zeiss microspectrophotofluorimeter (Laszlo, 1972). A direct relationship was found between the concentration of these monoamines and the intensity of fluorescence, when the latter was measured at the periphery of the droplets. The distribution of fluorescence intensity in these droplets was recorded and found to be fairly homogeneous. The colour of fluorescence of NA changes from green to yellowish green when its concentration increases, while the colour of fluorescence of 5-HT changes from green to yellow. The overlapping colour of fluorescence of these monoamines can be explained by their almost completely overlapping fluorescence spectra.

The large number of colours which occurred during these model experiments and in fluorescence microscopy raised the importance of an unambiguous description of