Microspectrofluorometric characterization of the fluorescent derivatives of biogenic amines produced by aqueous aldehyde (Faglu) fixation

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Received 6 July 1981

Summary

The fluorescent derivatives of the reaction between an aqueous aldehyde (Faglu) solution and the biogenic amines (5-hydroxytryptamine, dopamine and noradrenaline) have been examined in order to determine the conditions required for maximal fluorescence yield. The fluorescence intensity and spectra of the final reaction products have been characterized and found to be highly dependent on the pH of the reaction mixture. Fluorophores derived from catecholamines have maximal yield and are most easily characterized when the reaction is performed at pH 7.3, whilst those derived from 5-hydroxytryptamine have maximal yield and are most readily characterized when the reaction is performed at pH 10.0. The addition of potassium ferricyanide to the Faglu further enhances the fluorescence yield of 5-hydroxytryptamine-containing models and tissues at both pH 10.0 and pH 7.3. Using the modified Faglu reaction mixture, it has been possible to demonstrate 5-hydroxytryptamine in the central nervous system without the need for pharmacological manipulation.

Introduction

The production of fluorophores for the localization of biogenic amines following perfusion fixation with an aqueous solution of formaldehyde and glutaraldehyde (Faglu) has been described by Furness et al. (1977a,b). The technique enables fluorescence histochemistry and electron microscopy to be performed on the same samples and has a number of advantages over glyoxylic acid and formaldehyde vapour fixation (Furness et al., 1978). Furness et al. (1977b) applied the technique to aqueous models and obtained fluorescent products with adrenaline, noradrenaline, dopamine, dopa, 5-hydroxytryptamine (5-HT) and 5-hydroxytryptophan but not with histamine or octopamine. They also performed fluorescence spectrophotometry to determine the emission spectra of the fluorophores and these were similar to those of catecholamines and indole ethylamines determined by microspectrofluorometry.
Application of the Faglu technique gives a fluorescent product in models and 5-HT-containing enterochromaffin cells (Furness et al., 1977b) but not in the B1–B9 5-HT-containing perikarya of the brain stem (Blessing et al., 1978).

The work reported in this paper was undertaken to establish the optimal conditions for the reaction with Faglu and to characterize the fluorescent products of 5-HT, dopamine and noradrenaline in solid state models with the object of determining criteria for their recognition. A modified technique has been developed which is suitable for the demonstration of 5-HT in the central nervous system.

Materials and methods

The present study was undertaken using a modification of the Sephadex model previously described (Schofield & Wreford, 1979; Wreford & Smith, 1979, 1980). 10–20 mg of Sephadex G25 measuring 10–15 µm in diameter was allowed to swell at 4°C in 5 ml of a solution of 5-HT creatinine sulphate (1–10 mM), noradrenaline bitartrate (1–10 mM) or dopamine hydrochloride (1–10 mM) in 0.2 M phosphate buffer at pH 7.3 containing 0.1% glycine. In some experiments glycine was omitted and there was a considerable reduction in the fluorescence yield of the Faglu-treated models. After 4–6 h in the amine solution the suspensions were centrifuged at 1000 g for 5 min and the supernatant decanted. Approximately 10 ml of the appropriate formaldehyde–glutaraldehyde solution was added and the suspension was left to stand at 4°C for between 2 and 24 h. A smear of the suspension was then prepared on either a quartz or glass slide and dried for approximately 4 h in an evacuated desiccator over silica gel. The preparations were then mounted under immersion oil and a cover slip was applied. Faglu solutions were prepared in 0.2M phosphate buffer at pHs ranging from 5.0 to 8.0 and 0.2M carbonate-bicarbonate buffer at pHs ranging from 9.2 to 10.8. In some preparations potassium ferricyanide was added to the Faglu at concentrations varying from 0.1 to 0.8%. Because the ferricyanide is readily reduced to ferrocyanide, it is important to add it immediately before the Faglu is used. Final concentrations of fixative were 0.5% glutaraldehyde (TAAB, 25% solution, electron microscopy grade) and 4% formaldehyde (freshly prepared from paraformaldehyde). In some experiments glutaraldehyde was omitted and there was a substantial reduction in the fluorescence yield. Tissue was obtained from 2–3-month-old male Sprague-Dawley rats; some animals were given pargyline (200 mg/kg, i.p.) 2 h before killing. Following the administration of a Nembutal anaesthetic (50 mg/kg, i.p.) and heparin (4000 units/kg, i.p.), the thoracic cavity was opened, a cannula (17G) was inserted into the left ventricle and tied in place. The perfusion apparatus used was similar to that described by Furness et al. (1978), the blood was flushed from the animal with 150–200 ml of an ice-cold solution of phosphate (0.02 M) buffered normal saline containing 0.1% xylocaine; perfusion pressure was 120 mm Hg, the perfusion was then continued with ice-cold Faglu at the nominated pH. In some early experiments, 0.1% glycyne was incorporated in the Faglu solution. This resulted in an increase in the non-specific background fluorescence. After the passage of 400–500 ml of the perfusate, the brain was dissected out and placed in ice-cold fixative. The brain stem was cut into appropriately sized pieces, blotted dry and fixed to the stage of a Vibratome (Oxford Laboratories, California, U.S.A.) with an acrylic adhesive (Loctite; Loctite Corp., Newington, CT 06111, U.S.A.). Sections were cut serially at 30–40 µm and alternate sections were floated on to ice-cold fixative in a petri dish. The sections were mounted on to glass or quartz slides and dried in an evacuated desiccator over silica gel for approximately 4 h. In some experiments, phosphorous pentoxide was used as a desiccant but the fluorophores obtained after drying over silica gel were more intense and had more consistent