QUANTITATIVE CYTOCHEMICAL STUDIES ON CATECHOLAMINERGIC AND PEPTIDERIC NERVE TERMINALS

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FORMALDEHYDE INDUCED FLUORESCENCE OF CATECHOLAMINERGIC NERVE TERMINALS

Quantitative aspects

Catecholamines (CA) and indolamines (IA) can be visualized in histochemical preparations by the use of the formaldehyde induced fluorescence (FIF) method (Falck and Hillarp, 1962). In addition to morphological information on the localization, size and shape of monoaminergic neurones, this method can also provide information on the local concentration of CA and IA.

Theoretically, the FIF intensity is linearly related to the monoamine concentration in the preparation (for extensive discussion see Schipper and Tilders, 1982). This has been confirmed in studies on nonbiological models (Lichtensteiger, 1970; Einarsson, 1975; Schipper and Tilders, 1982). Also in a number of biological preparations that contain high concentrations of monoamines, such as mastcells (Enerback, 1975), pineal gland (Tilders et al. 1974), iris (Schipper et al. 1980c) and caudate nucleus (Einarsson, 1975), a good correlation was found between the FIF intensity and the monoamine concentration. Although quantitative cytochemistry does not allow interpretation in terms of absolute amounts of monoamines, the FIF intensity can be used as an index for changes in local monoamine content.

The advantage of quantitative FIF measurements compared to microchemical methods is the possibility to determine changes in amine content in specific brain structures that are too small to be dissected for microchemical analysis. For example, microfluorimetric measurements on FIF have revealed the existence of two dopamine systems in the median eminence (Lofstrom et al. 1976) and also two intermingling dopamine systems in the striatum with different turnover rates (Fuxe et al. 1978).

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Fig. 1 Left panel: Microphotograph of formaldehyde induced fluorescence in noradrenergic nerve terminals in the dilatatory muscle of the rat iris (whole mount preparation). Note the differences in dimensions and in fluorescence intensity of various varicosities.

Right panel: A: Fluorescence histogram obtained after scanning a 50 x 50 μm area in an iris preparation. B: calculated extraneuronal histogram (dark hatched). C: nerve fiber histogram (dark hatched) obtained by subtraction of the extraneuronal histogram from the total histogram. Abscissa: fluorescence intensity (arbitrary units). Ordinate: frequency expressed as percentage of total number of measurements (10000). Data from Schipper et al. (1980a).

Most quantitative fluorescence data have been obtained with "large field measurements", which involve measurements of the total fluorescence of large areas (100 - 1000 μm²) containing a large number of varicosities. Much more detailed information can be obtained by using scanning microfluorometry, which enables FIF intensity measurements in structures as small as individual varicosities. Within a terminal network, strong differences in FIF intensity between varicosities can be observed (see Fig. 1).

The functional relevance of these differences is unknown. In order to study the implications of this heterogeneity we have used the noradrenaline (NA) containing sympathetic terminal axons in the dilator muscle of the iris as a model. With the aid of a computer controlled fluorescence scanning microscope, large areas (50 x 50μm) in iris preparations have been scanned with a 0.5 μm