NEW PRINCIPLES FOR MICROSCOPIC DIFFERENTIATION BETWEEN DOPA, DOPAMINE AND NORADRENALINE

Letters to the Editor

Dopa and the primary amines, dopamine (DA) and noradrenaline (NA), are demonstrated with high sensitivity at the cellular level with the formaldehyde (FA) and the glyoxylic acid (GA) fluorescence histochemical methods (5, 6, 9, 10, 12, 13). However, the fluorophores formed from these substances show indistinguishable excitation and emission spectra. Dopa and DA cannot be differentiated in the fluorescence microscope (see references 3, 8 and 12), whereas after acidification of their FA-induced fluorophores dopa and DA can be distinguished from NA by microspectrofluorimetry (1, 2, 7). This differentiation procedure can usually be carried out only when the intensity of the intracellular fluorescence is rather high, and if this is not the case the method is usually quite laborious (see reference 2). Thus, there is obviously a great need for new methods for differentiation between dopa, DA and NA in the fluorescence microscope.

In a preliminary study (13) we found that in a combined FA and GA reaction, fluorophores with clearly different spectral properties can be formed from dopa and DA. In the present report the usefulness of the two-step reaction with FA and GA for differentiation between dopa, DA and NA has been evaluated.

The experiments were performed with the fluorogenic substances enclosed in histochemical protein droplet models. These were prepared by spraying 2% albumin solutions containing 0.5 mg/ml L-dopa, DA or NA on glass cover slips. The histochemical reactions were performed as follows. In the first step, the models were placed in 1-liter closed vessels containing 5 g paraformaldehyde, which either had been equilibrated in air of about 50% relative humidity (11) or excessively dried by heating at +100°C for 1 hr and subsequent storing over phosphorous pentoxide. The histochemical reactions were performed as follows. In the first step, the models were placed in 1-liter closed vessels containing 5 g paraformaldehyde, which either had been equilibrated in air of about 50% relative humidity (11) or excessively dried by heating at +100°C for 1 hr and subsequent storing over phosphorous pentoxide. The treatment was carried out at different temperatures (+4 to +10, +20, +50 and +80°C) for 10 min up to 3 hr. In the second step, the models were exposed to GA vapor at +100°C for 3 min with 300 torr GA in the reaction vessel as described by Lindvall and Björklund (12). Spectral analysis of the fluorophores was performed in a modified Leitz microspectrofluorometer (for details on the procedure see reference 4). Fluorescence intensities were evaluated subjectively in the fluorescence microscope. Blank values were obtained from identical albumin droplets free of added substances.

The DA fluorophore showed similar spectral characteristics after all combined treatments tested (Fig. 1A; excitation maxima at 330 and 375 nm; emission maximum at 470 nm). In contrast, either of two different types of fluorophore could be formed from dopa and NA, depending on the reaction conditions. One type of fluorophore showed excitation and emission spectra similar to those of the DA fluorophore (cf. Fig. 1A; excitation maxima at 330 and 375 nm; emission maximum at 470 nm). These spectral characteristics most probably indicate the formation of 3,4-dihydroisoquinolines similar to those formed in the FA or GA reaction alone (see Fig. 1C; references 6, 8, and 13). This type of fluorophore was formed with the more vigorous FA treatments (wet paraformaldehyde, higher temperature (+50°C, +80°C); under these conditions the models became strongly fluorescent already during the FA reaction. The other type of dopa and NA fluorophore had excitation maximum at 330 nm with a shoulder of varying size in the spectrum at about 360 nm (Fig. 1, A and B). The emission maximum was at 490-500 nm. Thus, the spectral characteristics of this type of fluorophore were clearly different from those of the DA fluorophore. This probably reflects the formation of fully aromatic isoquinolines from dopa and NA (see below and Fig. 1C). This second type of fluorophore was formed from dopa and NA when the first step (the FA treatment) was carried out under very mild reaction conditions, i.e., dry paraformaldehyde, low temperature (+4 to 10°C) and 30 min to 4 hr reaction time. After such FA treatments the models showed a very weak fluorescence, and a strong visible fluorescence developed during the subsequent GA treatment.

The present results clearly show that the combined FA and GA reaction allows the spectral differentiation both between dopa and DA, and between DA and NA. For spectral differentiation between NA and adrenaline (A) the combined FA and GA reaction, at
FIG. 1. A, excitation (left) and emission (right) spectra of the dopamine (----) and noradrenaline (------) fluorophores formed in a combined formaldehyde and glyoxylic acid reaction. Formaldehyde treatment: dry paraformaldehyde, +4°C, 30 min; glyoxylic acid treatment: 300 torr, +100°C, 3 min. B, excitation (left) and emission (right) spectra of the dopa fluorophore formed in a combined formaldehyde and glyoxylic acid reaction. Two examples of excitation spectra are represented, which illustrate the variable size of the shoulder at 360 nm. 'Treatment as above. The spectra are corrected instrument values and are expressed as relative quanta versus wavelength. C, proposed molecular structure of the fluorophores formed from dopa, dopamine and noradrenaline in the combined formaldehyde and glyoxylic acid reaction.

present, seems to offer no advantages over GA treatment alone. In both types of reactions NA and A give, in contrast to what is the case after FA treatment, clearly different excitation and emission spectra, but the fluorescence yield from A, which is very low in the GA reaction (cf. reference 12), is not markedly higher after combined FA and GA treatments.

The mechanisms of fluorophore formation from dopa in this procedure have been studied in some detail and are proposed to be as follows (13,14). In the initial FA reaction the low fluorescent tetrahydroisoquinoline-3-carboxylic acid is formed, and under the mild reaction conditions used no decarboxylation or fluorophore formation takes place. In the second step, the tetrahydro derivative reacts with GA forming a reactive 2-carboxymethylidihydroisoquinoline intermediate, which undergoes a further reaction with GA to yield 2,4-dicarboxymethylisoquinoline. The spectral characteristics of the NA fluorophore point to a molecular structure similar to that of the dopa fluorophore. The mechanisms of fluorophore formation for NA are not known in detail, but they probably proceed in a similar way as for dopa. The low fluorescent tetrahydroisoquinoline derivative formed in the first step is thus further reacted with GA in the second, yielding a fully aromatic isoquinoline. Whether GA only assists in the splitting off of the 4-hydroxyl group on the fluorophore molecule (forming a 2-carboxymethylisoquinoline as shown in Fig. 1C) or, in addition, reacts with the 4-carbon (forming a 2,4-dicarboxymethylisoquinoline) is unclear.

Interestingly, preliminary results have shown that other phenylethylamines and indolamines also can be differentiated from their corresponding amino acids by the combined FA and GA reaction. Moreover, with different amino acids the fully aromatic fluorophores are formed under different reaction conditions, which should offer new possibilities of distinguishing the individual substances. In this way, the combined FA and GA reaction greatly improves the possibilities for microspectrofluorometric identification of, and dis-

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tinction between, closely related fluorogenic compounds at the cellular level. Furthermore, the difference in emission peak maxima between the dopa and NA fluorophores and the DA fluorophore indicates a very attractive possibility of differentiation between these compounds directly in the fluorescence microscope by a readily distinguishable difference in color. These new principles are presently being applied to tissue for differentiation between intracellular dopa, DA and NA.

LITERATURE CITED

IMMUNOHISTOCHEMICAL LOCALIZATION OF SOMATOSTATIN IN THE RAT PANCREAS

Recently, it has been shown that somatostatin, a hypothalamic hormone which inhibits the release of growth hormone and thyrotropin (3, 4), can also inhibit the secretion of both insulin and glucagon (5, 10). Since this action of somatostatin has been shown in isolated dog and rat pancreas, a direct action of somatostatin on the endocrine pancreas has been postulated. The physiologic importance of somatostatin in the control of endocrine pancreatic secretion remains, however, to be established. The presence of somatostatin in the circulating blood or in the pancreatic tissue would suggest a role of somatostatin in the control of the secretion of insulin and glucagon. In order to accurately localize somatostatin in the pancreas, we used an immunohistochemical method at both the light and electron microscope levels.

Four male Sprague-Dawley rats weighing 250–300 g were perfused with 500 ml 4% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.4. The pancreas was subsequently dissected and fixed by immersion in the