DETECTION AND ASSAY OF DOPAMINE

ARVID CARLSSON

Department of Pharmacology, University of Lund, Lund, Sweden

The detection and assay of dopamine (3-hydroxytyramine or β-(3,4-dihydroxyphenyl)-ethylamine) has so far met with certain difficulties. Its pharmacological activity is generally much lower than that of adrenaline and noradrenaline, rendering bioassay troublesome. With ethylenediamine it forms a fluorescent condensation product (13) which, however, is hard to distinguish from the corresponding products of noradrenaline and, notably, adrenaline (9). Many years ago Dulière and Raper (6) showed that dopamine is easily oxidized to a red indole derivative, which in the absence of oxygen undergoes an intramolecular rearrangement to form 5,6-dihydroxyindole. The latter step is accelerated by the addition of alkali. In these respects dopamine thus behaves much like adrenaline, noradrenaline and dopa (3,4-dihydroxyphenylalanine). When the so-called trihydroxyindole method, which makes use of this principle, is applied in one of its present modifications to dopamine, however, the fluorescence produced is weak and almost indistinguishable from that derived from noradrenaline (8, 11). Dopamine has been assayed by applying both the ethylenediamine condensation and the trihydroxyindole reaction on the same sample. The difference between the values obtained by the two methods is then taken as a measure of the amount of dopamine present in the sample (10, 14). This procedure is somewhat cumbersome, and its specificity may not always prove satisfactory (7, 12); many catechol derivatives are known which form fluorescent products with ethylenediamine.

We have now observed that the trihydroxyindole method can be modified to become a sensitive and specific method for the detection and assay of dopamine (2, 4, 5). In this method the following four points are essential.

1) Dopamine is not so easily oxidized as adrenaline and noradrenaline, a relatively high concentration of ferricyanide or, preferably, iodine being necessary. The optimum pH for the oxidation is 6.5.

2) As an antioxidant during the rearrangement in alkali, sulfite is preferable to ascorbate, since the latter compound interferes with the final fluorescence.

3) After rearranging in alkali, pH should be adjusted to about 5.3 (by the addition of acetic acid). This step serves two purposes: a) the wavelengths of activation and fluorescence drop considerably, enabling differential estimation of dopamine in the presence of adrenaline and noradrenaline; and b) the fluorescence intensity increases.

4) After the final pH adjustment the spontaneous rise in fluorescence intensity is slow. It can, however, be accelerated by ultraviolet irradiation (Table 1).

The actual procedure is as follows. Adjust the pH of the sample to about 6.5. To a silica test tube add 1 to 3 ml sample (0.2 to 2 μg dopamine), 0.5 ml 0.1 M phosphate buffer (pH 6.5), water to give a total volume of 3.8 ml, and 0.05 ml 0.02 N iodine solution. After 5 minutes add 0.5 ml alkaline sulfite solution.

TABLE 1

Effect of ultraviolet irradiation on fluorescence intensity; dopamine concentration 0.5 μg/ml (as the hydrochloride)

Irradiation	Quality of Test Tube	Fluorescence Intensity, Arbitrary Units*	
min		*Read immediately*	*Read after 17 hours*
10	Silica	100†	100†
10	Pyrex glass	68	97
—	Pyrex glass	15–20‡	86

* Activating wavelength 345 mμ, fluorescent wavelength 410 mμ.
† Set to 100.
‡ Needle unsteady and rising during reading.

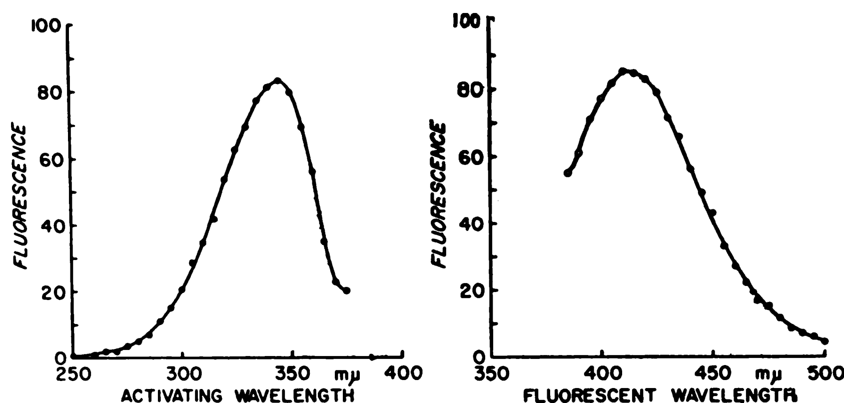

FIG. 1. Activation and fluorescence spectra of the fluorophore of dopamine.
Florescence is given in arbitrary units. When the activating wavelength was varied, the fluorescent wavelength was set to 410 mμ. When the fluorescent wavelength was varied, the activating wavelength was set to 345 mμ. Concentration of dopamine hydrochloride 0.5 μg/ml.

(Dissolve 5.04 g Na_2SO_3, 7 H_2O, in 10 ml water and dilute with 5 N sodium hydroxide to 100 ml.) After another 5 minutes add 0.6 ml 5 N acetic acid (pH drops to about 5.3). Irradiate sample by means of a mercury lamp for 10 minutes. (The lamp used in the present experiments was "Hanau NN 15/44 VK"; peak emission 254 mμ.) Read fluorescence in a spectrophotofluorometer. Activation and fluorescence peaks are 345 and 410 mμ, respectively. The fluorescence is stable for 24 hours.

A standard and a reagent blank are run together with the sample. When tissue extracts are analyzed, a "tissue blank" and an "internal standard" are also run together with the sample. The "tissue blank" is a sample treated as above, except that 5 N sodium hydroxide instead of the alkaline sulfite solution has been added; after the irradiation, sodium sulfite is added in the same amount as in the sample. The "internal standard" is a sample treated as above, except that a known amount of dopamine has been added.

Figure 1 shows the activation and fluorescence spectra of the fluorophore of

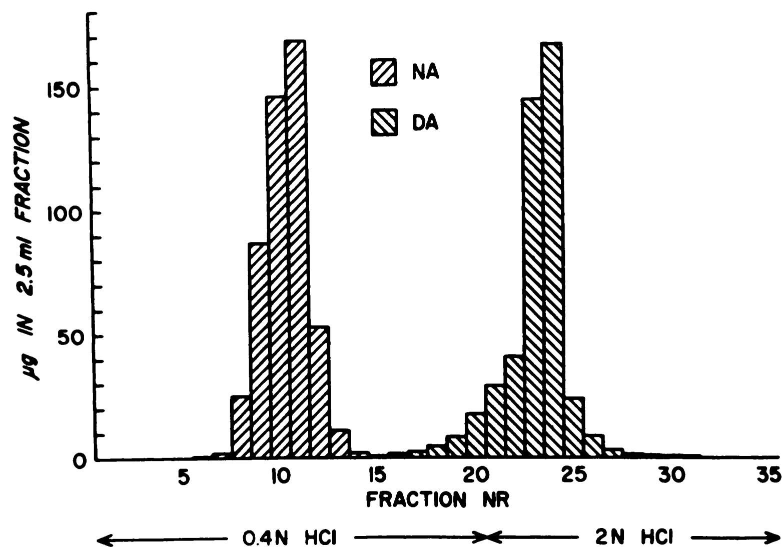

FIG. 2. Separation of noradrenaline (NA) and dopamine (DA) by ion-exchange chromatography.
Column: Dowex 50, Na^+, 20 mm^2 × 90 mm; eluant 0.4 and 2.0 N HCl.

dopamine. An Aminco-Bowman spectrophotofluorometer was used for the measurements. A linear relation exists between the concentration of dopamine and the fluorescence.

When added in amounts equal to that of dopamine, the following compounds have been found not to interfere with the determination of dopamine: adrenaline, noradrenaline, "metanephrine" (*i.e.*, adrenaline methylated in the 3-hydroxy position), "normetanephrine" (*i.e.*, noradrenaline methylated in the 3-hydroxy position), epinine (*i.e.*, N-methyl dopamine), tyramine, a number of other substituted phenylalkylamines, and 5-hydroxytryptamine. Dopa, on the other hand, yields a compound with fluorescence characteristics indistinguishable from those of the fluorophore of dopamine. If present, it must therefore be removed prior to assay. This is done, for example, when the extraction and purification procedure developed in our laboratory is used (1). Here the tissue to be examined is extracted with perchloric acid. The extract is neutralized with K_2CO_3 and passed through a Dowex 50 column, which should be in the sodium form. Adrenaline, noradrenaline and dopamine are taken up by the resin, while dopa passes through the column and is thus removed. The catecholamines are eluted with hydrochloric acid. Dopamine is held more strongly by the resin than adrenaline and noradrenaline and can thus be separated from these amines, if desirable (Fig. 2). The adrenaline and noradrenaline of the eluate are assayed essentially according to von Euler and Floding (8). Differentiation between adrenaline and noradrenaline is obtained by utilizing differences in activation spectra. The dopamine of the eluate is assayed as described here. For all three catecholamines the whole procedure has been analyzed in detail as regards recovery, accuracy and

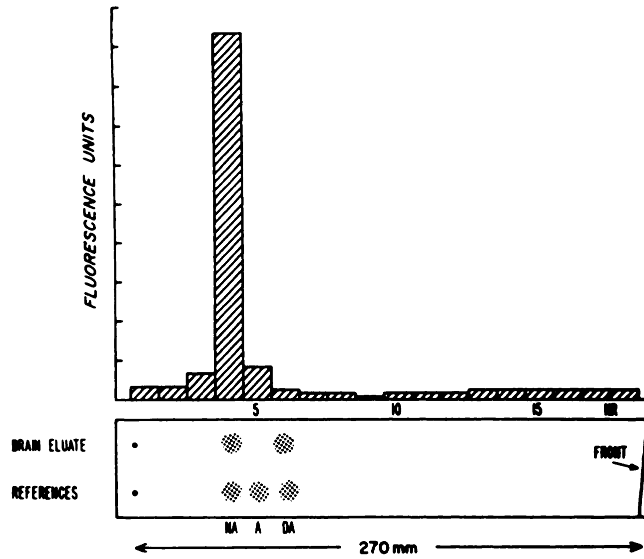

FIG. 3. Quantitative paper chromatography of an eluate from the rabbit brain. (Trihydroxyindole method.)

Note. The activation and fluorescence spectra obtained from paper number 4 were almost identical with those of noradrenolutine. The amount of noradrenaline recovered from the paper was 0.9 μg. The slight fluorescence, which is almost equally distributed in the majority of the samples is unspecific; it is due to the presence of an impurity in the paper.

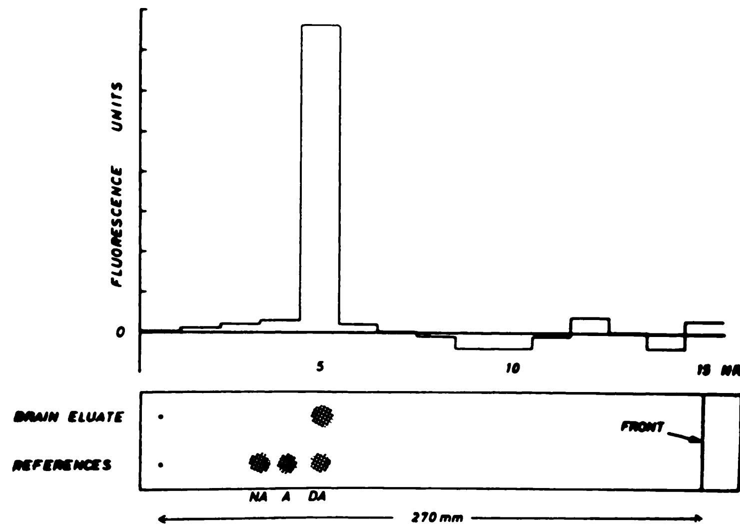

FIG. 4. Quantitative paper chromatography of an eluate from the rabbit brain. (Dopamine method.)

specificity, with satisfactory results. For example, recoveries from tissue extracts are generally at least 80%.

As an example of specificity tests some data of experiments on mammalian brain will be given. The activation and fluorescence spectra of the apparent noradrenaline in brain are indistinguishable from those of authentic noradrenaline. This holds true also of the R_f value, as determined by means of quantitative paper chromatography (butanol-HCl); the paper was cut into pieces which were separately eluted and assayed for noradrenaline (Fig. 3). The dopamine method has been analyzed in a similar manner. Thus the activation and fluorescence peaks of the apparent dopamine of the corpus striatum (cat), which contains most of the brain dopamine (3), are indistinguishable from those of authentic dopamine. The R_f value of the apparent dopamine of the rabbit brain is likewise indistinguishable from that of authentic dopamine (Fig. 4).

We have observed that not only dopamine, but also metanephrine and normetanephrine can be converted into strongly fluorescent derivatives when treated according to the principles of the trihydroxyindole method. The procedure differs in certain respects from those used for dopamine, noradrenaline and adrenaline. Consequently, metanephrine and normetanephrine do not interfere with the assay of the catecholamines as described here.

REFERENCES

1. Bertler, Å., Carlsson, A. and Rosengren, E.: A method for the fluorimetric determination of adrenaline and noradrenaline in tissues. Acta physiol. scand. **44**: 273–292, 1958.
2. Bertler, Å., Carlsson, A., Rosengren, E. and Waldeck, B.: A method for the fluorimetric determination of adrenaline, noradrenaline and dopamine in tissues. Kungl. fysiogr. sällsk. Lund förh. **28**: 121–123, 1958.
3. Bertler, Å. and Rosengren, E.: Occurrence and distribution of dopamine in brain and other tissues. Experientia **15**: 10–11, 1959.
4. Carlsson, A., Lindqvist, M., Magnusson, T. and Waldeck, B.: On the presence of 3-hydroxytyramine in brain. Science **127**: 471, 1958.
5. Carlsson, A. and Waldeck, B.: A fluorimetric method for the determination of dopamine (3-hydroxytyramine). Acta physiol. scand. **44**: 293–298, 1958.
6. Dulière, W. L. and Raper, H. S.: The tyrosinase-tyrosine reaction. VII. The action of tyrosinase on certain substances related to tyrosine. Biochem. J. **24**: 239–249, 1930.
7. Euler, U. S. von: Noradrenaline. Chemistry, Physiology, Pharmacology and Clinical Aspects, pp. 35, 94. Charles C Thomas, Springfield, Ill., 1956.
8. Euler, U. S. von and Floding, I.: A fluorimetric micromethod for differential estimation of adrenaline and noradrenaline. Acta physiol. scand. **33**: suppl. 118, 45–56, 1955.
9. Kägi, J., Burger, M. and Giger, K.: Modifikationen zur Fluorescenzmethode von Weil-Malherbe und Bone zur Bestimmung von Adrenalin und Noradrenalin im menschlichen Blutplasma. Arch. exp. Path. Pharmak. **230**: 470–478, 1957.
10. Montagu, K. A.: Catechol compounds in rat tissues and in brains of different animals. Nature, Lond. **180**: 244–245, 1957.
11. Shore, P. A. and Olin, J. S.: Identification and chemical assay of norepinephrine in brain and other tissues. J. Pharmacol. **122**: 295–300, 1958.
12. Valk, A. de T., Jr. and Price, H. L.: The chemical estimation of epinephrine and norepinephrine in human and canine plasma. I. A critique of the ethylenediamine condensation method. J. clin. Invest. **35**: 837–841, 1956.
13. Weil-Malherbe, H. and Bone, A. D.: The fluorimetric estimation of adrenaline and noradrenaline in plasma. Biochem. J. **67**: 65–72, 1957.
14. Weil-Malherbe, H. and Bone, A. D.: Intracellular distribution of catecholamines in the brain. Nature, Lond. **180**: 1050–1051, 1957.