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Note

Paper chromatographic separation of noradrenaline and its major metabolites

HENRYK MAJEWSKI and DAVID F. STORY
Department of Pharmacology, University of Melbourne, Parkville, Victoria 3052 (Australia)
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Investigations concerned with the release, uptake and metabolism of [^{14}C]- and [^{3}H]noradrenaline in animals, isolated tissues and in tissue homogenates usually require methods for the resolution of the total radioactivity into the unchanged radioactive catecholamine and its major metabolites: normetanephrine (NM); 3,4-dihydroxyphenylglycol (DOPEG); 3,4-dihydroxymandelic acid (DOMA); 3-methoxy-4-hydroxymandelic acid (VMA) and 3-methoxy-4-hydroxyphenylglycol (MOPEG). At present chromatographic techniques would appear to offer the most convenient means of achieving this separation. The paper chromatographic (PC) techniques previously utilized[1,2] require that the assay samples be divided and chromatographed in at least two separate solvent systems: to our knowledge, no single solvent system has so far been reported which allows complete separation of these compounds.

This paper describes a simple PC method using a single solvent system which yields total separation of noradrenaline and its major metabolites. The system is also capable of fully resolving adrenaline and its five major metabolites and yields a partial separation of dopamine and its major metabolites.

EXPERIMENTAL

Materials

The following compounds were obtained from Sigma (St. Louis, Mo., U.S.A.): (—)noradrenaline hydrochloride; (±)normetanephrine hydrochloride; 3,4-dihydroxyphenylglycol; 3,4-dihydroxymandelic acid; (±)3-methoxy-4-hydroxymandelic acid; bis(3-methoxy-4-hydroxyphenylglycol) piperazine; (±)metanephrine hydrochloride; 3,4-dihydroxyphenylacetic acid and dopamine hydrochloride. The following compounds were obtained from Calbiochem (Los Angeles, Calif., U.S.A.): (—)adrenaline-bitartrate and 3-methoxy-4-hydroxyphenylethanol. The 3-methoxy-4-hydroxyphenylethylamine was obtained from K & K Labs. (Plainview, Texas, U.S.A.). Whatman chromatography paper was obtained from Reeve Angel. All reagents used were of analytical grade.

Tritiated noradrenaline, (—)7-[^{3}H]noradrenaline, was obtained at a specific activity of 3.8 Ci/mmole from New England Nuclear (Boston, Mass., U.S.A.); its radiochemical purity was stated as 97.0%. Tritiated dopamine, 3,4-dihydroxyphenyl-

ethylamine (ethyl-2-[^{3}H] was obtained from New England Nuclear. The specific activity was 7.5 Ci/mmole and the radiochemical purity was stated as 99.0%.

Methods

In one series of experiments the separation of noradrenaline and its major metabolites, NM, DOPEG, DOMA, MOPEG, and VMA, was tested. In another series of experiments adrenaline and its major metabolites, metanephrine (MN), DOPEG, DOMA, MOPEG, and VMA, were tested for separation and in a third series of experiments dopamine and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC), 3-methoxy-4-hydroxyphenethanol (MHPE), 3-methoxy-4-hydroxyphenylacetic acid (HVA), and 3-methoxy-4-hydroxyphenylethylamine (MHPA), were tested for separation.

Pure non-radioactive samples of the compounds were dissolved in solutions containing disodium ethylenediaminetetraacetic acid (1.7 m*M*) and ascorbic acid (5.7 m*M*). The test compounds were applied as single spots to Whatman No. 1 paper (3 × 45 cm) either individually (2-μl aliquots of 10 m*M* solutions), or as mixtures (5-μl aliquots of solutions containing the required compounds each at a concentration of 2 m*M*). Where [^{3}H]noradrenaline or [^{3}H]dopamine was chromatographed, 0.03 μCi of either compound was applied to the paper in 2 μl of a carrier solution containing the non-radioactive catecholamine and its metabolites each in a concentration of 2 m*M*. Spots were applied 7.5 cm up from the bottom of the chromatography paper and allowed to dry at room temperature.

The solvent system used was: *n*-butanol–ethyl acetate–glacial acetic acid–7% (w/v) sulphur dioxide solution–90% (w/w) formic acid–10 *M* hydrochloric acid (200:170:90:140:5:10). The tank containing the solvent mixture was lined with Whatman No. 3 paper which had been previously soaked in the solvent. After being sealed the system was allowed to equilibrate at least 45 min at 4° before adding the chromatogram. A sheet of Whatman No. 1 paper equal in size to the chromatogram was folded and clipped to the top of the chromatogram before placing it in the tank.

The chromatograms were run in an ascending form until the solvent had moved about 30 cm from the origin (18–24 h). They were then allowed to dry at room temperature for 1 h and the compounds were visualized by spraying the chromatograms with 20% Folin-Ciocalteu's reagent followed by exposure to ammonia vapour for a few minutes[5]. In experiments in which tritiated catecholamines were chromatographed the separated compounds were not visualized, but after drying, the chromatograms were divided into 3-mm horizontal strips between the origin and the solvent front. The strips were then placed in vials containing 1 ml of 1 *M* hydrochloric acid and 10 ml of liquid scintillation solution of the following composition: 5.5 g of 2,5-diphenyloxazol (PPO); 0.1 g of 1,3-bis-2-(5-phenyloxazolyl)-benzene (POPOP) and 333 ml of Triton X-100 made up to 1 lin toluene. The vials were shaken vigorously and left standing for at least 1 h at room temperature. Radioactivity (counts/min) was measured in a Packard model 3380 Scintillation counter. Counting efficiency as determined by automatic external standardisation (AES) ratio ranged from 25 to 27%.

RESULTS AND DISCUSSION

Using the method described, total separation of noradrenaline and its five

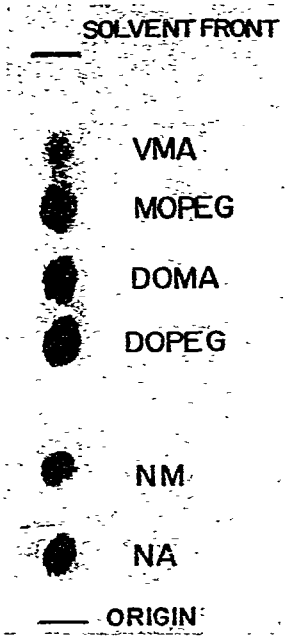

Fig. 1. Ascending paper chromatogram of noradrenaline (NA) and its metabolites normetanephrine (NM), 3,4-dihydroxyphenylglycol (DOPEG), 3,4-dihydroxymandelic acid (DOMA), 3-methoxy-4-hydroxyphenylglycol (MOPEG) and 3-methoxy-4-hydroxymandelic acid (VMA) using *n*-butanol–ethyl acetate–glacial acetic acid–7% (w/v) sulphur dioxide solution–90% (w/w) formic acid–10 *M* hydrochloric acid (200:170:90:140:5:10) as the solvent.

major metabolites was achieved. A photograph of a developed chromatogram is shown in Fig. 1 and the R_F values of each compound are given in Table I. Adrenaline and its five major metabolites were also adequately separated using the solvent system described as shown by a photograph of a developed chromatogram in Fig. 2. The R_F values of adrenaline and its metabolites are given in Table I. However, dopamine

TABLE I

R_F VALUES OF NORADRENALINE, ADRENALINE, DOPAMINE AND THEIR RESPECTIVE MAJOR METABOLITES

Paper, Whatman No. 1; solvent, see text (Methods).

Compound	R_F	*Compound*	R_F	*Compound*	R_F
Noradrenaline	0.18	Adrenaline	0.21	Dopamine	0.26
Normetanephrine	0.32	Metanephrine	0.36	3-Methoxy-4-hydroxy-phenylethylamine	0.37
3,4-Dihydroxyphenyl-glycol	0.52	3,4-Dihydroxyphenyl-glycol	0.52		
3,4-Dihydroxymandelic acid	0.63	3,4-Dihydroxymandelic acid	0.63	3,4-Dihydroxyphenyl-acetic acid	0.75
3-Methoxy-4-hydroxy-phenylglycol	0.74	3-Methoxy-4-hydroxy-phenylglycol	0.74	3-Methoxy-4-hydroxy-phenylacetic acid	0.86
3-Methoxy-4-hydroxy-mandelic acid	0.83	3-Methoxy-4-hydroxy-mandelic acid	0.83	3-Methoxy-4-hydroxy-phenethanol	0.84

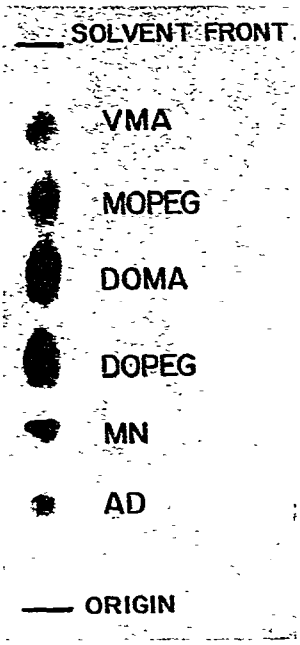

Fig. 2. Ascending paper chromatogram of adrenaline (AD) and its metabolites metanephrine (MN), 3,4-dihydroxyphenylglycol (DOPEG), 3,4-dihydroxymandelic acid (DOMA), 4-methoxy-4-hydroxyphenylglycol (MOPEG) and 3-methoxy-4-hydroxymandelic acid (VMA). Solvent, as in Fig. 1.

and its major metabolites were incompletely separated by this solvent although dopamine itself was separated from all of its metabolites. The R_F values for dopamine and each metabolite are also given in Table I.

To test whether the compounds retained their homogeneity in the solvent system, [^{3}H]noradrenaline was chromatographed with non-radioactive noradrenaline and the five noradrenaline metabolites. In these experiments, 97.1% [standard error of the mean (S.E.M.) 1.1%; $n = 3$] of the total radioactivity recovered from the developed chromatograms was located on the spot corresponding to authentic non-radioactive noradrenaline. Similarly, when [^{3}H]dopamine was chromatographed, together with non-radioactive dopamine and its metabolites, 98.8% (S.E.M. 0.7%; $n = 3$) of the radioactivity on the papers coincided with the location of authentic dopamine.

As suggested by the R_F values in Table I, the solvent system should also be capable of separating dopamine from noradrenaline. This was verified by chromatographing [^{3}H]dopamine and [^{3}H]noradrenaline together with the non-radioactive catecholamines and their metabolites. The tritium was then recovered largely in the peaks with R_F values corresponding to those for dopamine and noradrenaline as shown in Fig. 3.

Thus, the method described provides a simple, single-solvent system for the full resolution of either noradrenaline or adrenaline and their respective metabolites. It may also be used to separate dopamine from noradrenaline, and provides a partial separation of dopamine and its metabolites. The system was convenient to use and

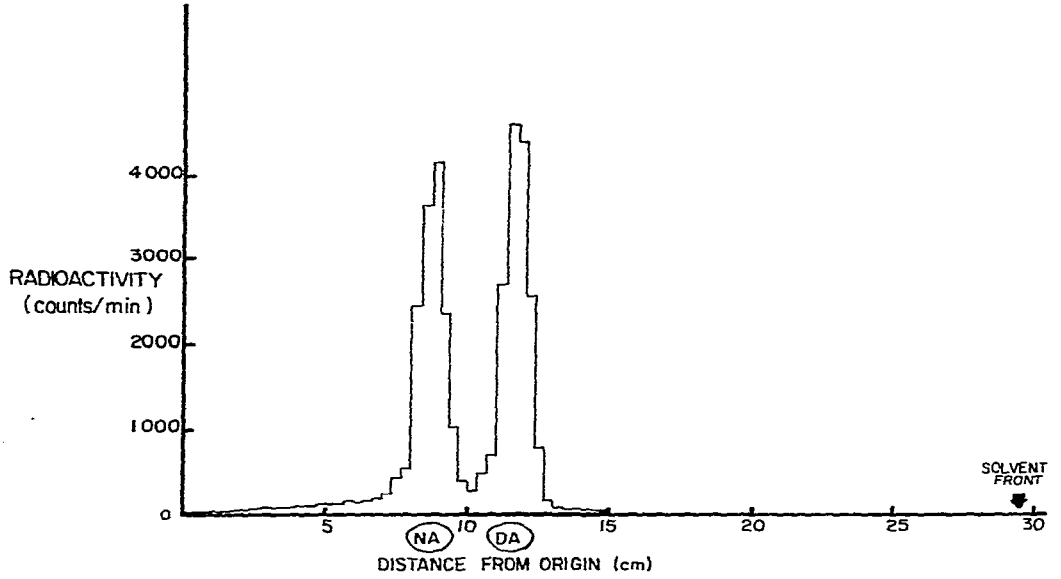

Fig. 3. Distribution of radioactivity on an ascending paper chromatogram of [^{3}H]noradrenaline and [^{3}H]dopamine. Solvent, as in Fig. 1. The positions of authentic noradrenaline (NA) and dopamine (DA) are indicated.

the chromatograms could be run overnight. However, it should be noted that, if the prepared solvent system was kept for longer than 24 h, it tended to separate into two phases. It is therefore recommended that the solvent system be freshly prepared and used only once.

ACKNOWLEDGEMENTS

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