RESEARCH PAPERS

PAPER CHROMATOGRAPHY OF SOME TISSUE AMINES

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When solutions of adrenaline, noradrenaline, histamine, 5-hydroxytryptamine and related amines in trichloroacetic, trifluoroacetic or picric acid are chromatographed in various organic solvent systems, the active material divides into two areas. If a basic amino acid is included in the solution before chromatography, the separation becomes more complete. It is suggested that the second area consists of a loose complex between the amine and the corresponding acid. When solutions of the amines in hydrochloric, acetic or oxalic acid are chromatographed, the active material resides only in one area.

WHEN extracts of animal tissues are tested for some of their biologically active constituents, usually they are freed from blood and other protein, often by treatment with trichloroacetic acid. Occasionally such extracts are also subjected to paper chromatography. When the chromatograms are developed the active areas are sometimes found to be split into two. For example, the two spot-formation was reported in 1952 for adrenaline when extracts of adrenal glands were made in this way and chromatography was carried out in various organic solvents[1]. In a similar manner, histamine was found in 1954 to reside in two distinct areas when extracts of mast cell tumours were chromatographed[2]. The present work is a further study of this phenomenon and includes the testing of extracts of tissues rich in 5-hydroxytryptamine (5-HT) or its derivatives.

METHODS

Solutions (0·1 per cent, w/v) of pure synthetic amines possessing adrenaline-like, histamine-like or 5-HT-like properties were made either in 0·33 N hydrochloric acid, or in 5 per cent trichloroacetic, trifluoroacetic, picric, acetic or oxalic acids. In some experiments, arginine, lysine or ornithine (1 per cent w/v) were also included in the solutions. Ten μg. of each amine were applied to paper (Whatman No. 1) and ascending chromatograms run for 18 hours. The solvents used were *n*-butanol: acetic acid: water (4 : 1 : 5), *iso*propanol: ammonia: water (20 : 1 : 2), *n*-butanol saturated with N HCl, and *n*-propanol: water (1 : 1·5).

The spray developers included aqueous potassium iodate[3], alkaline potassium ferricyanide and formaldehyde[4], *p*-nitroaniline diazo reagent[5], and Folin and Ciocalteu reagent to detect adrenaline-like substances, Pauly diazo-reagent, chiefly for detecting histamine, and Ehrlich's and N.N.C.D. reagents, to detect 5-HT and its derivatives.

For testing the biological activity of certain locations on the paper, chromatograms were made in duplicate, one paper being developed by the spray reagent whilst the other was used for elution of the active areas

with 0·01N HCl. The eluates were then tested on the blood pressure of a spinal cat for adrenaline-like activity, on the isolated ileum of the guinea pig for histamine, or on the isolated atropinised uterus of a rat in oestrus for 5-HT-like activity. In some experiments, extracts of tissues rich in adrenaline, noradrenaline, histamine, 5-HT or tryptamine were similarly used.

RESULTS

Adrenaline and Related Compounds

A solution of adrenaline in hydrochloric acid showed only one adrenaline spot at $R_F = 0·32$ when chromatographed in the butanol: acetic acid: water solvent, whereas a similar solution in 5 per cent trichloroacetic acid showed two active areas at $R_F = 0·32$ and 0·63. When each area from the latter chromatogram was eluted and assayed for adrenaline, the activity was almost equally divided between the two areas. If the excess trichloroacetic acid was removed from the solution by ether extraction before chromatography, the intensity of the faster-running spot ($R_F = 0·63$) was greatly reduced. That the effect was entirely due to the presence of trichloroacetic acid was shown by passing the solution through a column of the anion exchange resin, Amberlite IRA-400, chloride form. The filtrate containing adrenaline in hydrochloric acid, approximately 0·33N, gave only one spot on chromatography at $R_F = 0·32$. Little or no loss in activity had occurred on passage of the solution through the resin. Since weakly acid phenols may form rather unstable compounds with trichloroacetic acid[6], it has been suggested that the additional faster-running spot is caused by such a complex formation between adrenaline and trichloroacetic acid[1]. The material in the faster-running area readily split up for simple elution of this area followed by rechromatography in the same solvent resulted in a single adrenaline spot at $R_F = 0·32$. A trichloroacetic acid spot was then also detected at $R_F = 0·90$ with a potassium iodide-iodate-starch developer.

The double-spot phenomenon with adrenaline also occurred when the other solvent systems were used. Solutions of adrenaline in trifluoroacetic or picric acids likewise yielded double spots of adrenaline, but those in oxalic, acetic or hydrochloric acid did not. When a basic amino acid such as lysine, arginine or ornithine was also present in the solution of adrenaline in trichloroacetic, trifluoroacetic or picric acid, more than 80 per cent of the adrenaline activity passed to the faster-running area ($R_F = 0·63$).

The double-spot formation was similarly observed with solutions of the following adrenaline-like substances. (*a*) Dihydroxyphenylethanolamines and their α-substituted derivatives, such as noradrenaline, *N*-ethylnoradrenaline, *N*-*iso*propylnoradrenaline (isoprenaline), corbasil and α-ethylnoradrenaline, (*b*) dihydroxyphenylethylamines, such as dopamine and epinine, (*c*) monohydroxyphenylalkylamines such as tyramine, paredrine, *p*-sympatol and *m*-sympatol, (*d*) phenyl*iso*propanolamines such as propadrine and ephedrine, (*e*) the phenyl*iso*propylamine, amphetamine,

(*f*) the ketone, adrenalone, and (*g*) the amino acids, 3,4-dihydroxyphenylserine and 3,4-dihydroxyphenylalanine. The formation of double spots of noradrenaline and of each of the amino acids was achieved only when the amount of substance applied to the paper was increased at least 10 times, that is, to more than 100 μg. In all cases, the presence of a basic amino acid facilitated the separation of the active area into two and increased the amount found in the faster-running area.

When an extract of cat suprarenal glands in trichloroacetic acid was similarly chromatographed, the adrenaline was found to reside in two distinct locations (R_F = 0·32 and 0·64). Likewise, an extract of a canine phaeochromocytoma showed on chromatography two distinct areas for noradrenaline (R_F = 0·25 and 0·62). In this experiment, relatively large concentrations of both arginine and lysine were also detected on the chromatograms; these two basic amino acids no doubt aided the separation of the noradrenaline into two areas under the conditions used.

Histamine

When solutions of histamine in hydrochloric, acetic or oxalic acid were applied to paper and chromatograms were run in the butanol: acetic acid: water solvent, a compact spot of histamine was formed at R_F = 0·11. However, solutions in trichloroacetic, trifluoroacetic or picric acid treated similarly showed two concentrated areas of histamine at R_F = 0·11 and 0·65, with some trailing between the two locations. About 10 per cent of the biological activity resided in the faster-running area. As with adrenaline, the presence of a basic amino acid facilitated the formation and increased the intensity of the area at R_F = 0·65 so that 60 per cent of the activity resided there, whereas partial removal of the trichloroacetic acid by ether extraction reduced its intensity. When an eluate of the faster-running area was re-run in the same solvent, the histamine now remained entirely in its lower location (R_F = 0·11). Similar results were obtained with the other organic solvents used.

Extracts of tissues exceptionally rich in histamine, such as ox pleura, ox liver capsule, and mast cell tumours from dogs, behaved similarly to solutions of pure histamine and showed two areas of histamine on chromatography. In these experiments, relatively large concentrations of arginine were also detected on the chromatograms and no doubt aided the separation of the histamine into two areas. Recently, the two-spot formation of histamine has been noted when extracts of liver of a case of urticaria pigmentosa were subjected to chromatography[7].

Compound 48/80, a potent histamine-liberator *in vivo*, was next included in the mixture of histamine and trichloroacetic acid which was applied to the paper. This was done when it was found that the only amino acids to facilitate the formation of the faster-running area of histamine were the basic ones which had already been shown to be the only amino acids capable of releasing histamine from tissues[8]. In fact, compound 48/80 (20 μg.) completely separated the histamine (10 μg.) into the two areas, with more than 75 per cent of the histamine activity in the faster-running location. But tubocurarine, another histamine-liberator *in vivo*,

in amounts up to 2 mg. for every 10 μg. of histamine, failed to increase the histamine activity of the faster-running area and even did not remove the trail between the two histamine locations. Nevertheless, when both compound 48/80 or tubocurarine and a basic amino acid were contained in the mixture before chromatography, complete separation of the histamine occurred and over 80 per cent of the activity passed to $R_F = 0{\cdot}65$. The amounts of compound 48/80 or tubocurarine needed for this effect were as little as 3 μg. Two other histamine-liberators, ammonia and sodium hydroxide (0·1N), also effectively separated the histamine activity into two locations when added to solutions of histamine in trichloroacetic acid before chromatography.

5-Hydroxytryptamine

Solutions of 5-HT in trichloroacetic, trifluoroacetic or picric acid gave the two-spot formation when chromatographed in the solvents listed. In the butanol: acetic acid: water solvent system, for example, the 5-HT positions were at $R_F = 0{\cdot}38$ and 0·64, with about 30 per cent of the total activity residing in the faster-running area. When a basic amino acid was included in the mixture before chromatography, more than 70 per cent of the 5-HT activity passed to the area of $R_F = 0{\cdot}64$. When this area was eluted and the eluate re-run in the same solvent, a single spot characteristic of free 5-HT ($R_F = 0{\cdot}38$) was produced. Solution of 5-HT in acetic, oxalic or hydrochloric acid gave only one spot of 5-HT ($R_F = 0{\cdot}38$). When an extract of a carcinoid tumour (producing much 5-HT) was made in trichloroacetic acid and a chromatogram prepared as for the pure 5-HT solutions, there were two distinct locations of 5-HT ($R_F = 0{\cdot}36$ and 0·60), the fast-running area containing over 60 per cent of the 5-HT activity. Extracts made in acetone showed only one spot ($R_F = 0{\cdot}38$) when chromatographed similarly.

The formation of two spots of 5-HT also occurred in the other solvents used. Similarly, solutions of tryptamine or *NN*-dimethyl-5-HT (bufotenine) in trichloroacetic acid showed two distinct areas of each amine. Likewise, an extract of tomatoes[9] in trichloroacetic acid gave two tryptamine spots.

Discussion

The remarkable ability of trichloroacetic, trifluoroacetic or picric acid to extract biologically active amines from tissues may be related not only to their coagulative effect on protein but also to their participation in a loose complex which contains the amine and generally one or more basic amino acids. Detailed analyses of the tissue extracts used in the present experiments show that relatively large concentrations of either arginine or lysine or both are present, and these most probably play a part in deciding whether the active material resides in one or two areas on the chromatograms. Another factor is the concentration of the acid used, for its partial removal causes most of the active amine to be found in its normal location on the chromatogram. But, it can be shown that the two-spot formation does not occur when aqueous solvents (such as

8 per cent w/v sodium chloride) are used for chromatography. These observations therefore emphasise the need for great care in the preparation and interpretation of chromatograms of biologically active amines extracted from tissues.

In any one solvent system used in the present experiments, the R_F values of the faster-running spots of adrenaline, noradrenaline, histamine or 5-HT are similar (0·60 to 0·66). This suggests that for each of these amines a similar unstable complex is formed. Since these complexes on elution are physiologically active, they may have some biological importance.

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