Individual elution of noradrenaline (together with adrenaline), dopamine, 5-hydroxytryptamine and histamine from a single, strong cation exchange column, by means of mineral acid-organic solvent mixtures

Few methods have been published which demonstrate the quantitative measurement of the biogenic amines noradrenaline, adrenaline, dopamine, 5-hydroxytryptamine (5-HT) and histamine, and their metabolites, in the same, small sample of tissue after a single extraction and purification procedure. Recently Sadavongvivad (1970) published a technique employing butanol in the organic extractions of catecholamines, and of 5-HT together with histamine, from the same, small sample of tissue. We have developed a column adsorption chromatographical procedure which permits the total amount of each amine, derived from the tissue, to be concentrated into small, individual fractions.

Noradrenaline together with adrenaline has been separated from dopamine on strong cation exchange columns of dimensions 50 mm (in buffer) by 4·2 mm (i.d.). The resin used is Dowex 50W–X4, 200–400 mesh, sodium form (Bertler, Carlsson & Rosengren, 1958, as later modified by Carlsson & Lindqvist, 1962). Noradrenaline, together with adrenaline, is eluted with 8 ml, and dopamine with the following 12 ml, aqueous N HCl. Adopting the procedures of Kahlson, Rosengren & Thunberg (1963) and of Green & Erickson (1964), we were able to elute histamine with 5 ml of aqueous 2N HCl after eluting the catecholamines.

Using large volumes (up to 20 ml) of eluant, 5-HT could be eluted after histamine with 4–6N aqueous HCl or 0·01N aqueous NaOH, the latter being an adaptation of the technique of Wiegand & Scherfling (1962). The eluate volume could be reduced to 4 ml by eluting with 3N ethanolic (50%) HCl, when adopting the procedure of Schildkraut, Schanberg & others (1969).

Whilst the procedure so far described permits the separation of all the amines, with the exception of noradrenaline from adrenaline, some further interesting observations and improvements were made. In common with 5-HT, the elution of noradrenaline, adrenaline and dopamine is also greatly facilitated by the use of certain

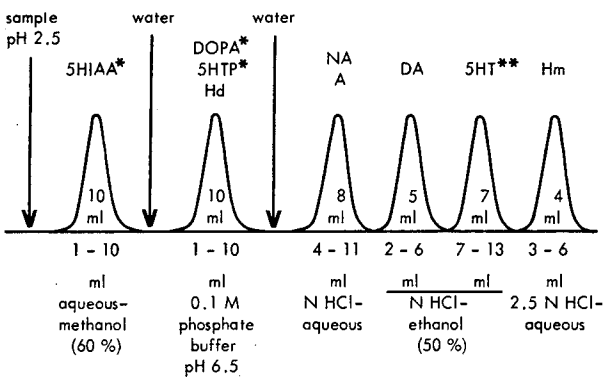

FIG. 1. Order of elution of noradrenaline (NA) (together with adrenaline, A), dopamine (DA), 5-hydroxytryptamine (5-HT) and histamine (Hm) and their respective precursors (dihydroxyphenylalanine–DOPA, 5-hydroxytryptophan, 5-HTP and histidine, Hd) and 5-hydroxyindole acetic acid (5-HIAA), from a strong cation exchange column (of dimensions 72 mm in buffer by 4·0 mm i.d. Dowex^{R} 50W–X4, 200–400 mesh, sodium form)

(* According to Lindqvist.)

(** The volume of 5-HT eluate can be reduced to 3·5 ml by eluting with 1·8N HCl-ethylene glycol monoethyl ether (ethyl cellosolve) (50%), whilst still permitting the subsequent, separate elution of Hm.)

Table 1. *Recovery from columns of dopamine* (DA) *and 5-hydroxytryptamine* (5-HT), *when added to rat intestinal extracts**

Sample number	No addition to tissue extract* DA μg	5-HT μg	Addition of amines to respective tissue extract DA μg	5-HT μg	Recovery of added amines DA μg (%)	5-HT μg (%)
1	3·906	—	5·000	—	4·808 (96%)	—
2	9·472	3·256	10·000	3·000	9·405 (94%)	2·530 (84%)
3	3·469	5·426	5·000	5·000	4·517 (90%)	3·980 (80%)
4	0·034	7·145	0·050	6·000	0·053 (107%)	5·155 (86%)
5	13·190	3·719	10·000	3·000	9·889 (99%)	2·657 (89%)
6	3·345	4·317	3·000	5·000	2·714 (90%)	4·184 (84%)
7	isotonic saline solution		1·000	1·000	1·062 (106%)	0·970 (97%)

* Tissue obtained from rats being used in an L-DOPA administration experiment; Atack, Enerbäck & Häggendal—unpublished.

organic solvents mixed with the HCl, but the elution of histamine is almost unaffected.

The procedure which we have adopted as the most promising is represented diagrammatically in Fig. 1. If the sample is loaded onto the column at pH 2·5, the corresponding amino-acids are also adsorbed (Bertler & others, 1958; Wiegand & Scherfling, 1962; Kahlson & others, 1963). Dr. M. Lindqvist found that 5-hydroxyindole acetic acid (5-HIAA) may interfere with the assay of 5-hydroxytryptophan (5-HTP). Consequently, Lindqvist has developed a technique in this laboratory for the elution of 5-HIAA using aqueous methanol (60%) and for 5-HTP, together with dihydroxyphenylalanine (dopa), using 0·1M phosphate buffer, pH 6·5, from a column similar to ours. We have observed histidine to be eluted together with the other amino-acids. The subsequent elution procedure for the amines is unaffected by the methanol and buffer eluants.

The amines (and amino-acids and 5-HIAA) can all be assayed spectrophotofluorometrically. The assays of noradrenaline and adrenaline according to e.g. Bertler & others (1958) or Häggendal (1963), and of histamine according to Green & Erickson (1964), require little modification. Histamine is eluted with 2·5N aqueous HCl because the presence of ethanol or cellosolve was found to lower the sensitivity of the assay. The unlikely possibility of interference by spermidine is being examined. The assay of dopamine by the dihydroxyindole technique has been slightly but significantly modified and specificity tests are being undertaken.

5-HT has been assayed according to the technique of Andén & Magnusson (1967). These authors noted a slow decrease in fluorescence after irradiation, a tendency which appears to be accentuated in the presence of the organic solvents used.

The major changes described above are in the elution patterns of dopamine and 5-HT. In a short experiment to determine the progress of the method, the recoveries of dopamine and 5-HT were recorded and are presented in Table 1.

The procedure as outlined permits the individual separation of the total tissue content of noradrenaline (together with adrenaline), dopamine, 5-HT and histamine (and their respective amino-acid precursors and 5-HIAA). In addition, dopamine is eluted in the much smaller volume of 5 ml (as opposed to 12 ml), without using a stronger acid. Both factors will significantly increase the overall sensitivity of the assay of the amines.

Organic solvents dissolved in aqueous eluting media, to differentially alter the elution behaviour of adsorbed compounds, have previously been used in the separation of inorganic substances. Their successful use in the present study, whereby the elution behaviour of the monoamines, especially 5-HT, was changed but not that of histamine, suggests a wider application to the differential separation of compounds of biological interest.

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Inhibitory effect of propranolol on the vasoconstrictor response to sympathetic nerve stimulation

Propranolol enhances the vasoconstrictor effect of noradrenaline in skeletal muscle (Brick, Hutchinson & Roddie, 1967, Shanks, 1967), spleen (Ross, 1967a) and the mesenteric circulation (Ross, 1967b), an effect thought to be a consequence of inhibition of the effect of noradrenaline on vasodilator β-adrenergic receptors. Whether propranolol, through the same mechanism, may also enhance the vasoconstrictor response to sympathetic nerve stimulation has received less attention. Burks & Cooper (1967) did find propranolol to increase the vasoconstrictor responses both to exogenous noradrenaline and to sympathetic nerve stimulation in canine isolated perfused mesenteric arteries. We have compared the effect of propranolol on the vasoconstrictor responses to peripheral sympathetic nerve stimulation and to intra-arterially injected noradrenaline in the hind leg of the anaesthetized cat.

Cats, 2·5–3·1 kg, were anaesthetized with pentobarbitone sodium, eviscerated, and the lumbar sympathetic chain on one side cut at L3-L4 and a bipolar electrode placed on the distal part of the nerve. The femoral artery on the same side was catheterized in both directions, and the blood flow to the leg passed through a constant-flow Sigmamotor pump. The perfusion pressure to the leg, recorded by means of a Statham transducer on an Offner Dynograph, was initially adjusted to correspond to the systemic arterial pressure. The blood flow to the paw was occluded by means of a tight ligature.

The sympathetic nerves were stimulated for 90 s with impulses of supramaximal voltage, 4 ms duration and a frequency (1–2 impulses/s) (Grass S4 stimulator) that produced an increase of perfusion pressure of 50–80 mm Hg. Nerve stimulations were alternated according to a standardized time schedule with intra-arterial injections of noradrenaline in a dose (0·25–1 μg) that also increased the perfusion pressure 50–80 mm Hg. When stable responses to nerve stimulation and injected noradrenaline had been established, propranolol was infused intravenously during 5 min in a dose of 0·1 mg/kg, followed after 45 min by another infusion of 0·5 mg/kg propranolol. Three responses to each of the vasoconstrictor stimuli were recorded after each dose of