J. Pharm. Pharmacol., 1965, **17**, 429–433 Received March 5, 1965

Estimation of dopamine, noradrenaline, adrenaline and 5-hydroxytryptamine from single rat brains

GEORGE BROWNLEE AND T. L. B. SPRIGGS

A solvent extraction method was used to extract dopamine, noradrenaline, adrenaline and 5-hydroxytryptamine from single rat brains. Spectrophotofluorimetric estimations of dopamine, noradrenaline, adrenaline and 5-hydroxytryptamine were completed 5½ hr after beginning the extraction procedure. The method was applied to the routine estimation of brain amines.

PUBLISHED methods for the extraction and estimation of dopamine from tissue extracts usually involve the use of chromatographic columns and are time consuming. This disadvantage is avoided by applying the solvent extraction method devised by Shore & Olin (1958) for noradrenaline, to the extraction of dopamine. Since in addition to noradrenaline and dopamine, adrenaline and 5-hydroxytryptamine (5-HT) are also extracted by this method, they may be estimated in the same extract from a single rat brain. This paper describes the method and establishes its authenticity and reliability.

Experimental

METHODS

Male Wistar rats, 180–260 g, were killed by stretching the neck; the brains were dissected, the meninges and the cerebellum removed and the remainder stored at −10°.

Extraction of brain amines. A single rat brain was dropped into liquid nitrogen or liquid air, shattered (Callingham & Cass, 1963) and dispersed in 30 ml of butanol reagent to which was added 2 ml 0·01N hydrochloric acid and 5 g sodium chloride. The suspension was shaken for 60 min in a 70 ml stoppered bottle to extract the brain amines into the butanol phase. After centrifugation at 2,000 rev/min for 5 min, 25 ml of the butanol layer was withdrawn and added to 50 ml n-heptane + 7 ml 0·01N hydrochloric acid. Shaking for 5 min sufficed to pass the amines from the organic phase into the aqueous phase, and after centrifugation at 2,000 rev/min for 2 min, of the aqueous phase recovered, 6·5 ml was distributed for assay as follows: 2·5 ml for the dopamine assay, 1·5 ml for the noradrenaline assay, 1·5 ml for the adrenaline assay, and 1·0 ml for the 5-HT assay.

Assay of dopamine. (Derived from the method of Carlsson & Waldeck, 1958). To 2·5 ml of the 0·01N hydrochloric acid extract is added 0·5 ml of 0·1M phosphate buffer (pH 6·5). The pH is then adjusted to 6·46–6·54 by dropwise addition of 0·5M and 0·1M potassium carbonate whilst bubbling with nitrogen. Adjustment of the pH is critical (pH was measured with a pH meter using micro-electrodes). Aliquots of 2 × 1·2 ml are placed in 4 inch × $\frac{5}{8}$ inch test tubes (one for test, one for blank) and to each is added 0·05 ml of the iodine-potassium iodide solution.

From the Department of Pharmacology, King's College London, Strand, W.C.2.

After 5 min, 0·5 ml alkaline sulphite solution is added to the test and 0·5 ml 2·5N sodium hydroxide is added to the blank. After a further 5 min, 0·6 ml of 2·5N acetic is added to both test and blank and these are then irradiated vertically under an ultraviolet lamp (Phillips TL20 W/8) for 20 min, when 0·05 ml water is added to the test and 0·05 ml M Na_2SO_3 solution is added to the blank. The fluorescence is measured in a spectrophotofluorimeter, with an excitation wavelength of 335 mμ and a fluorescence wavelength of 378 mμ (uncorrected values).

Assay of noradrenaline, adrenaline and 5-HT. Noradrenaline and adrenaline were estimated by the method of Shore & Olin (1958) but using one quarter of the volume of all reagents specified in their text.

5-HT was estimated by the method of Udenfriend, Weissbach & Bogdanski (1955).

Drugs. Dopamine hydrochloride; adrenaline hydrochloride; noradrenaline bitartrate; 5-hydroxytryptamine creatinine sulphate; phenelzine sulphate; amitriptyline hydrochloride; reserpine.

Reagents. A.R. grade reagents were used unless noted. The water used throughout was triple glass-distilled.

Butanol reagent: 500 g of sodium chloride was shaken with a mixture of 4 litres of n-butanol (D.C.L. Biscol) and 0·7 litres 0·01N hydrochloric acid. Excess acid was removed and the saturated butanol allowed to stand for 24 hr when any further acid was removed. The reagent was stored over sodium chloride. *n-Heptane* (Phillipps, 99 mol %, or B.D.H.). 0·1M *Phosphate buffer* pH 6·5: 1·075 g $Na_2HPO_4.12H_2O$ + 0·952 g KH_2PO_4 was made up to 100 ml with water. *Iodine-potassium iodide solution:* 0·085 g iodine + 1·67 g potassium iodide were dissolved in 5 ml water and diluted to 100 ml with water. *Alkaline sulphite solution:* 2·52 g $Na_2SO_3.7H_2O$ was dissolved in 10 ml of water, 10 g sodium hydroxide was added and water to 100 ml. 2·5N *Acetic acid:* 14·25 ml glacial acetic acid was made up to 100 ml with water.

Results

The relation between the concentration and the fluorescence intensity of dopamine developed by this method is linear, the points falling on a straight line within the range 0·01 and 1·28 μg/ml (Fig. 1). That dopamine is extracted quantitatively by the extraction procedure is shown in Fig. 2. When pure dopamine solutions are extracted, 75% of the dopamine is recovered in the final acid aliquot.

When dopamine is added to rat brain tissue before extraction, 72% $\pm$3·8% s.e. (14 experiments) of that added is recovered in the final acid aliquot. Similarly, the recovery from rat brain tissue of added noradrenaline is 59% $\pm$1·6% s.e. (14 experiments) and of added 5-HT is 87% $\pm$3·2% s.e. (14 experiments).

Noradrenaline, adrenaline and 5-HT do not contribute significantly to the fluorescence developed by an equal weight of dopamine. The fluorescence intensities developed by equal weights of base are: dopamine 100·0; dopa 74·0; noradrenaline <0·1; adrenaline 0·2; 5-HT <0·1. Although dopa fluoresces under these conditions, its interference in

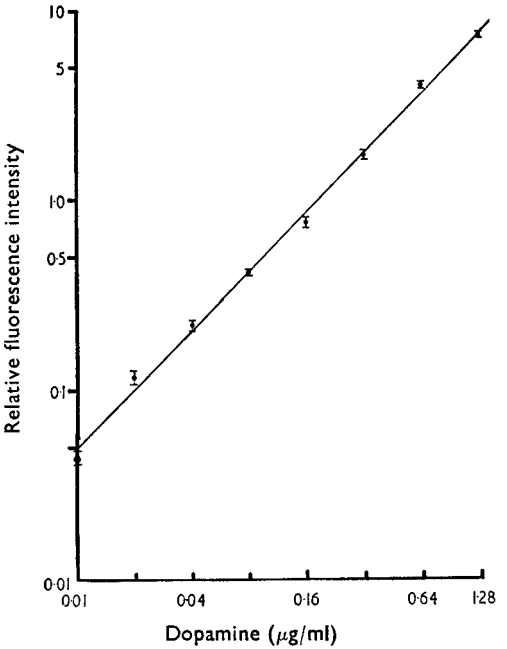

FIG. 1. The relation between fluorescence intensity developed and concentration of dopamine. Each point is the mean from 6 experiments, and the vertical lines represent the s.e. of the mean.

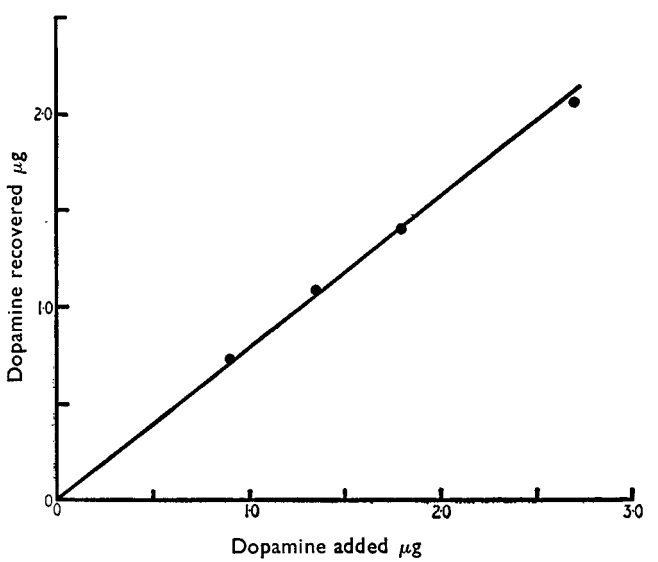

FIG. 2. Graph showing the quantitative extraction of dopamine by this method. Each point is the mean from three experiments.

tissue extracts is negligible, since the amount present in most tissues is small (less than 0·04 μg/g, Anton & Sayre, 1964).

The excitation and fluorescence spectra of rat brain extracts which have been subjected to the dopamine assay procedure are shown in Fig. 3.

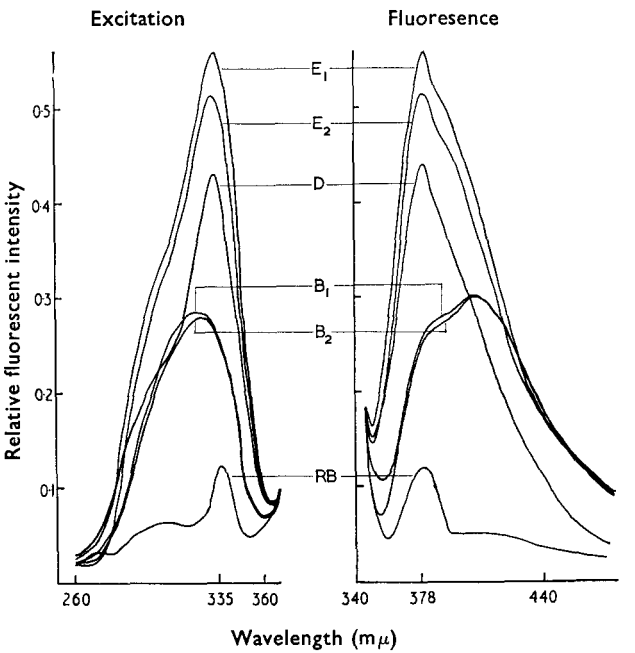

FIG. 3. Excitation and fluorescence spectra of extracts from rat brain (E_1 and E_2), tissue blanks (B_1 and B_2), pure dopamine 0·1 μg/ml (D) and a reagent blank (RB).

The spectra peaks from the brain extracts correspond to the spectra peaks of pure dopamine. The scatter peaks for all samples are similar and the small peaks in the reagent blank spectra coincide with the wavelengths calculated for Raman scatter.

The fluorescence intensity of blanks from brain extracts is reproducible and consistent; the peaks are larger than those of a reagent blank but are broad and non-specific compared with the dopamine peaks. With an extract from a single rat brain there is sufficient difference between the test and the blank readings to allow precise estimates of the dopamine content. The values obtained for the absolute content of dopamine, noradrenaline, adrenaline and 5-HT in rat brain are in agreement with values previously published in the literature (Table 1).

APPLICATION OF THE METHOD

The application of the method was challenged by examining the changes of brain catecholamine levels in the rat after treatment with drugs known to influence these substances. Thus after treatment with the monoamine oxidase inhibitor, phenelzine, 30 mg/kg intraperitoneally, the levels of

TABLE 1. COMPARISON OF VALUES FOR AMINE CONTENTS OF RAT BRAIN OBTAINED BY DIFFERENT WORKERS

Method	Amine content as μg/g fresh brain				Authors
	Dopamine	Noradrenaline	Adrenaline	5-HT	
Column separation methods	0·62 ± 0·03*	0·39 ± 0·01	—	0·51 ± 0·01	Gey & Pletscher (1961)
	0·60	0·49	—	—	Carlsson (1959)
	0·68	0·32	0·04	—	Anton & Sayre (1964)
	0·54 ± 0·04	0·42 ± 0·01	—	—	Moore & Lariviere (1963)
Present method	0·68 ± 0·05	0·40 ± 0·02	<0·01	0·59 ± 0·01	Brownlee & Spriggs

* Denotes s.e. of mean. The sign — means not reported.

dopamine, noradrenaline and 5-HT were all increased 4 hr after injection. After reserpine, 1 or 2 mg/kg, the characteristic reduction in all three amines was found; in addition the effects were dose dependent. Amitriptyline, however, did not alter the dopamine, noradrenaline or 5-HT content of the rat brain. These results are shown in Fig. 4 in which each histogram represents the mean from three rat brains.

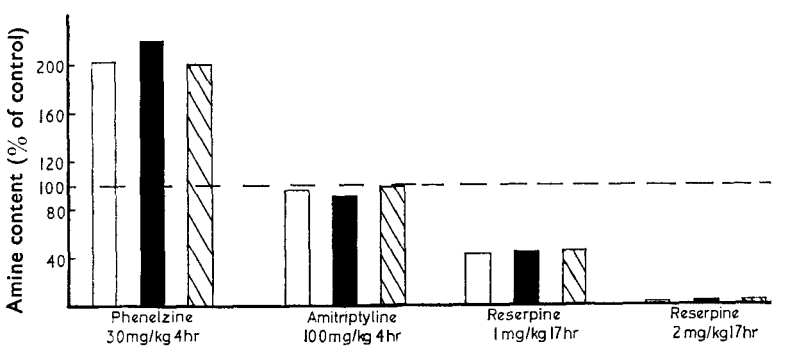

FIG. 4. The effects of phenelzine, reserpine or amitriptyline on the amine content of rat brain. Dopamine (solid columns), noradrenaline (open columns) and 5-HT (hatched columns) levels are increased after phenelzine, reduced after reserpine and unmodified after amitriptyline. Each histogram represents the mean from three rat brains.

Because of the fluorescence arising from non-specific substances extracted from tissues, the method, when applied to dopamine, is unsuitable for the estimation of the absolute amounts in tissues, when these fall below about 0·15 μg/g.

References

Anton, A. H. & Sayre, D. F. (1964). *J. Pharmacol.*, **145**, 326–336.
Callingham, B. A. & Cass, R. (1963). *J. Pharm. Pharmacol.*, **15**, 699–700.
Carlsson, A. (1959). *Pharmacol. Rev.*, **11**, 300–304.
Carlsson, A. & Waldeck, B. (1958). *Acta physiol. scand.*, **44**, 293–298.
Gey, K. F. & Pletscher, A. (1961). *J. Pharmacol.*, **133**, 18–24.
Moore, K. E. & Lariviere, E. W. (1963). *Biochem. Pharmacol.*, **12**, 1283–1288.
Shore, P. A. & Olin, J. S. (1958). *J. Pharmacol.*, **122**, 295–300.
Udenfriend, S., Weissbach, H. & Bogdanski, D. F. (1955). *Science*, **122**, 972–973.