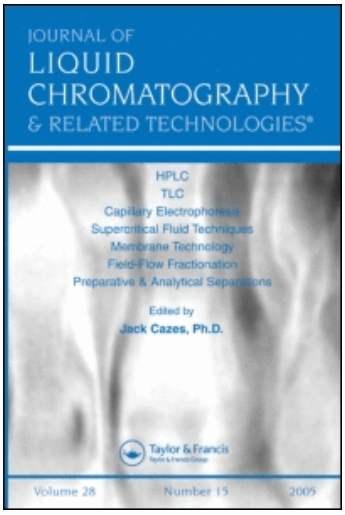

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HPLC Determination of Monoamines in Rat Brain after Enzymatic Treatment with Ascorbate Oxidase and Sulfatase

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HPLC DETERMINATION OF MONOAMINES IN RAT BRAIN AFTER ENZYMATIC TREATMENT WITH ASCORBATE OXIDASE AND SULFATASE

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ABSTRACT

The simultaneous analysis of norepinephrine, dopamine and serotonin along with their respective metabolites, MHPG, DOPAC and 5HIAA is accomplished in rat brain by using a recently developed HPLC technique. Sulfated MHPG is decongegated with sulfatase for detection purposes and endogenous ascorbate is diminished with ascorbate oxidase to reduce the front.

INTRODUCTION

In refining our HPLC techniques for the simultaneous detection of several monoamines in mouse brain (1,2), ascorbate oxidase was added to reduce the size of the front (3,4). However, when this technique was applied to the rat brain, the levels of ascorbate were so high that the front obscured the loci where both NE and MHPG should elute in the chromatogram. Moreover, since MHPG is largely sulfated in the rat brain, it cannot be properly analyzed in this species without prior enzymatic hydrolysis (5).

Because of these problems, several investigators have foregone measurement of MHPG in rat brain tissue (6-9). This has prevented the accurate assessment of the noradrenergic system under the experimental conditions selected. In the present study, we are reporting successful application of HPLC to the analysis of monoamines in rat brain, including MHPG.

METHODS

The present HPLC methodology and its application to mouse brain tissue have been reported in detail previously (1-3) but modifications of the extraction procedure were needed for the rat brain. The concentration of ascorbate oxidase was increased to 2 mg/ml due to the higher levels of ascorbate in rat brain. In addition, we employed sulfatase Type H-5 (Sigma, St. Louis, MO) in order to liberate conjugated MHPG into its free form so that it could be detected under our chromatographic conditions. We have followed the sulfatase method of Warnoff (5) but with a higher concentration of sulfatase (15 mg/ml).

The brains were weighed, homogenized, and extracted in 200 µl of a sodium acetate buffer pH 5.0 (3.0g sodium acetate and 4.3 ml glacial acetic acid per liter) containing the internal standard (20 ng/ml IP). The homogenate was filtered through Isolab micro-columns (Akron, Ohio) during centrifugation (2,300 G/5 min). The filtrate was diluted in a 1:1 ratio with the extraction medium and divided into two aliquots. One aliquot was incubated with 25 µl sulfatase at 37°C for 3 hours, and then cooled in an ice bath for one minute. The other aliquot did not receive sulfatase. Ascorbate oxidase was added to both aliquots 45 minutes prior to the first injection so that the reaction could go to completion (at 4°C).

RESULTS AND DISCUSSION

The chromatogram illustrating the standard run is depicted in Fig. 1. It demonstrates the resolution of eleven compounds during a period of less than twenty minutes. Note that NE is the first

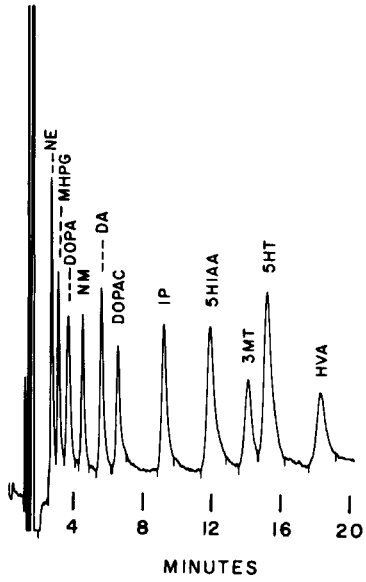

FIGURE 1
STANDARDS

compound to emerge and that NE is followed directly by MHPG. In Fig. 2, an ascorbate oxidase treated brain sample (septum), without sulfatase, is illustrated to demonstrate the separation of NE from the front and the absence of detectable levels of MHPG and DOPAC. Five compounds are detected and resolved. In Fig. 3, samples treated with sulfatase reveal the additional presence of MHPG (as a distinct, measurable peak behind NE) and DOPAC.

In Table 1, the values for the compounds analyzed in septal tissue are given in ng/gm tissue before and after sulfatase hydrolysis. MHPG appears in the sulfatase treated samples in the amount of 80 ± 6.3 ng/gm tissue.

Note also that several other compounds treated with sulfatase are altered. This may be due in part to sulfate decongegation but some of the differences may be due to metabolism caused by the

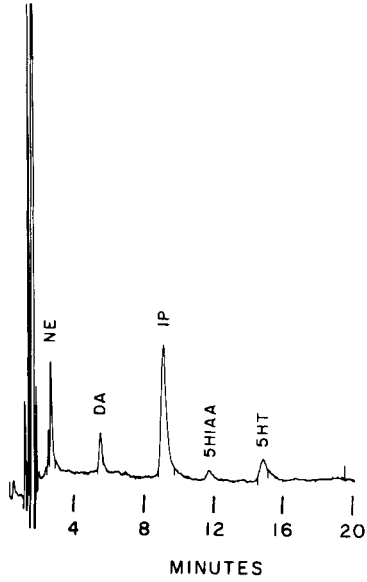

FIGURE 2
WITHOUT SULFATASE TREATMENT

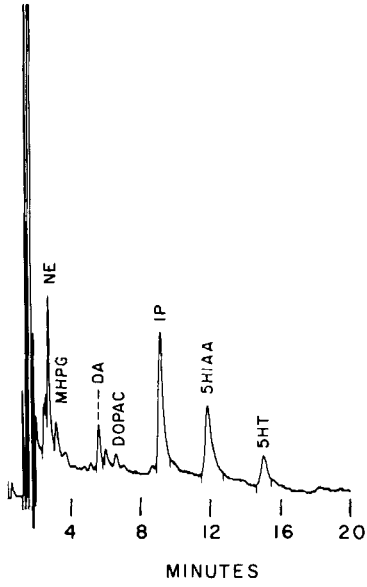

FIGURE 3
WITH SULFATASE TREATMENT

TABLE 1

COMPOUNDS	NE	MHPG	DA	DOPAC	5HT	5HIAA
Without Sulfatase Treatment	280 ±20	- -	143 ±13	- -	54 ±11	31 ±5.3
With Sulfatase Treatment	240 ±30	80 ±6.3	101 ±13	42 ±5	82 ±8.8	248 ±18

incubation. Therefore, one may wish to utilize the preincubation values for reporting purposes, except for MHPG, and to standardize the post-incubation MHPG values by reducing them to predicted pre-incubation levels. This can be accomplished by using the ratio of the pre-incubation and post-incubation NE values as a reduction factor. Nevertheless, Warnhoff (5) reports the post-incubation values for all compounds and this results in complete uniformity. He also provides sensitivity and selectivity values for the present sulfatase technique (5).

In conclusion, this HPLC method for detection of monoamines and metabolites in rat brain tissue, including MHPG, should prove helpful where the simultaneous analysis of noradrenergic, dopaminergic and serotonergic compounds is desired under identical experimental conditions in a short period of time.

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