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Note

Determination of monoamine oxidase B activity by high-performance liquid chromatography

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Monoamine oxidase (MAO; monoamine O_2:oxidoreductase, EC 1.4.3.4) is the enzyme which catalyses the oxidative deamination of monoamines. It has a broad substrate specificity and it plays a major role in the catabolism of monoamines in the central nervous system. Two forms of MAO, designated MAO A and MAO B, have been identified both in vivo and in vitro in animal brain [1]. They are demonstrable by their preference for different amines as substrates and by their sensitivity to specific inhibitors. MAO A preferentially deaminates norepinephrine and 5-hydroxytryptamine and is selectively inhibited by clorgyline. MAO B deaminates benzylamine and β-phenylethylamine and is sensitive to inhibition by deprenyl and pargyline [2].

MAO activity can be determined by a large number of different techniques. These include spectrophotometry [3, 4], fluorometry [5, 6], polarographic monitoring of oxygen consumption [7], and measurement of ammonia [8] or hydrogen peroxide [9] formed in the reaction. The most frequently used are radiochemical assays because of their specificity and sensitivity [10—12]. However, radiochemical assays require time-consuming extractions and use of rather expensive isotopes.

High-performance liquid chromatography (HPLC) is an accurate and a sensitive method to assay many of the catecholamine-metabolizing enzymes [13]. MAO A activity and non-specific MAO activity have been determined using HPLC with fluorescence detection [14]. This communication describes a fast, simple assay for MAO B activity using benzylamine as substrate, deproteinization with perchloric acid and detection of the product benzaldehyde at 254 nm.

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EXPERIMENTAL

Reagents

Benzylamine hydrochloride and benzaldehyde were obtained from EGA-Chemie (Steinheim, F.R.G.). Pargyline was from Sigma (St. Louis, MO, U.S.A.). Clorgyline was purchased from May & Baker (Dagenham, U.K.). Methanol, HPLC grade, was from Orion Pharmaceutical Co. (Espoo, Finland). Sodium heptane sulphonic acid was from Eastman-Kodak (Rochester, NY, U.S.A.). All other reagents were of analytical grade and purchased from commercial sources.

Sample preparation

Female rats weighing about 200 g were killed with carbon monoxide. Brains were quickly removed and homogenized in four volumes of cold 0.9% potassium chloride. Homogenates were kept in small aliquots at −20°C until assayed. Protein determinations were carried out using a Bio-Rad protein assay kit (Bio-Rad Labs., Richmond, CA, U.S.A.).

Assay

The enzyme incubation mixture contained the following components in a total volume of 0.5 ml: 0.35 ml of 0.2 *M* sodium phosphate buffer pH 7.2, 0.1 ml of brain homogenate and 0.05 ml of 2 m*M* benzylamine. The mixture was incubated for 30 min at 37°C except for the study of time course. The reaction was stopped by the addition of 50 μl of 4 *M* perchloric acid. Protein was removed by centrifugation. A 20-μl aliquot was injected into the liquid chromatograph.

Chromatography

The modular liquid chromatographic system consisted of a Waters Model 6000A pump, a Waters Intelligent Sample Processor (WISP) Model 710 B (Waters Assoc., Milford, MA, U.S.A.), a 150 × 4.6 mm, 5-μm Ultrasphere-ODS column fitted with a 45 × 4.6 mm precolumn (Beckman Instruments, Fullerton, CA, U.S.A.). The eluted components were detected by ultraviolet (UV) absorption at 254 nm with a Waters Model 441 fixed-wavelength detector. The elution was carried out isocratically at ambient temperature using 40% methanol containing 50 m*M* sodium phosphate and 1 m*M* heptane-sulphonic acid. The pH was adjusted to 3.2 with sodium hydroxide. The flow-rate was 1.5 ml/min. The enzyme activity was calculated as nmoles benzaldehyde formed per min per mg protein.

RESULTS AND DISCUSSION

Fig. 1 shows typical chromatograms obtained with crude rat brain homogenate. The blank (Fig. 1A), i.e. homogenate without substrate, shows that no interfering peaks are present in the described assay system. The retention time for the product benzylaldehyde is 5.2 min (Fig. 1B). It can be further decreased down to 3 min by increasing methanol and phosphate concentrations or omitting the ion-pairing reagent. The assay system was developed

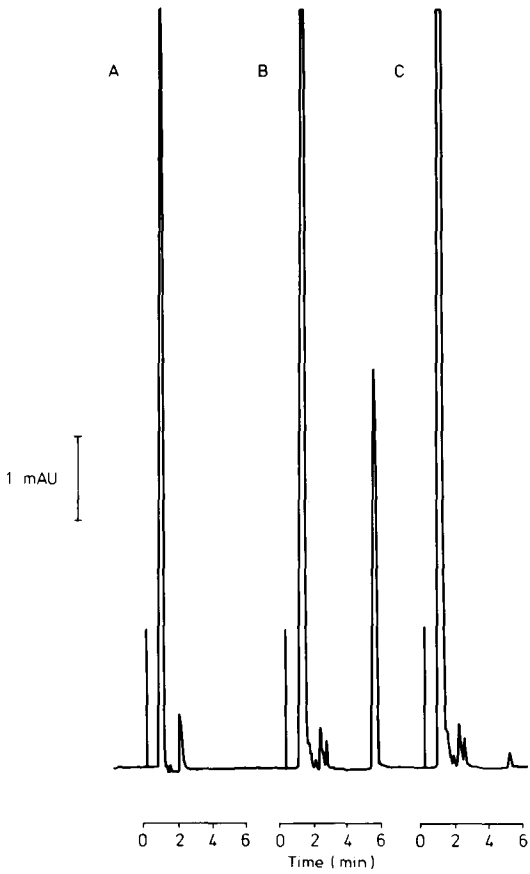

Fig. 1. Chromatograms obtained with MAO preparations: (A) blank, i.e. rat brain homogenate incubated without benzylamine; (B) homogenate incubated with 0.2 m*M* benzylamine; (C) homogenate incubated with 0.2 m*M* benzylamine and 10 n*M* pargyline. Chromatographic conditions: 5-μm Ultrasphere-ODS column (4.5 × 150 mm); mobile phase, 40% methanol in 50 m*M* sodium phosphate buffer, pH 3.2, containing 1 m*M* sodium heptanesulphonic acid; flow-rate, 1.5 ml/min; detection at 254 nm; sensitivity, 0.01 a.u.f.s., injection volume, 20 μl.

mainly for rapid screening of MAO B inhibitors, which would have interfered with the product in other elution systems.

Since the inhibition of MAO activity with pargyline decreases the size of the peak with retention time corresponding to benzaldehyde (Fig. 1C), it can be concluded that no endogenous compounds are present in the system. It also confirms the specific and sensitive inhibition of MAO B activity by pargyline. The activity was not diminished in the presence of 10 n*M* clorgyline which selectively inhibits MAO A [10].

The calibration curve indicates a linear relationship between the peak height and the amount of benzaldehyde from 0.2 to 20 nmol/ml. The detection limit with signal-to-noise ratio of 5:1 was 150 pmol/ml, making it possible to detect very low MAO B activities.

The rate of aldehyde formation expressed as growing height of the benzaldehyde peak on the liquid chromatogram showed a linear relationship $Y =$

$9.27X + 0.47$, $r = 0.996$ ($n = 3$), with up to 60 min of incubation time. The standard error between data points was less than 3.0%. Linearity was also demonstrable between the amount of enzyme preparation for 10—250 μg protein and enzymatically formed benzaldehyde during 30 min of incubation at 37°C. It is described by the equation $Y = 0.13X + 0.33$ with $r = 0.994$ and C.V. < 5.0% between data points ($n = 3$).

The reproducibility of the assay using the standard procedure ($n = 6$) was 2.5%, expressed as coefficient of variation. This precision was achieved without the use of an internal standard and illustrates the excellent properties of HPLC techniques for accurate enzyme activity determinations. Since the sample recovery is 100%, no internal standard is required.

The MAO B activity in whole rat brain ($n = 6$) with 0.2 m*M* benzylamine as substrate was 1.28 ± 0.03 nmol/min/mg protein. This value is in close agreement with the values obtained by radiochemical assay [15].

MAO B activity has recently been assayed also using gas chromatography (GC). However, these techniques require derivatization procedures before the products of MAO reaction can be determined. The derivatization of hydrogen peroxide for GC is fast but produces high blanks [16], while the derivatization of aldehydes for GC is slow [17].

The described HPLC method is precise, sensitive and rapid, allowing the handling of about sixty samples per day and thus making it useful for rapid screening of MAO B activity in different tissues.

REFERENCES

1 J.P. Johnston, Biochem. Pharmacol., 17 (1968) 1285.
2 J. Knoll and K. Magyar, Adv. Biochem. Psychopharmacol., 5 (1972) 393.
3 C.W. Tabor, H. Tabor and S.M. Rosenthal, J. Biol. Chem., 208 (1954) 645.
4 H. Weissbach, T.E. Smith, J.W. Daley, B. Witkop and S. Udenfriend, J. Biol. Chem., 235 (1960) 1160.
5 K.F. Tipton, Anal. Biochem., 28 (1969) 318.
6 K. Zaitsu, H. Nagai, K. Kohashi and Y. Ohkura, Chem. Pharm. Bull., 25 (1977) 918.
7 D.R. Weetman and A.J. Sweetman, Anal. Biochem., 41 (1971) 517.
8 H. Yamada, H. Suzuki and Y. Ogura, Adv. Biochem. Psychopharmacol., 5 (1972) 185.
9 H.Y. Meltzer and S.M. Stahl, Res. Commun. Chem. Pathol. Pharmacol., 7 (1974) 419.
10 J.A. Roth and K. Feor, Biochem. Pharmacol., 27 (1978) 1606.
11 C. Mitra and S.R. Guha, Biochem. Pharmacol., 29 (1980) 1213.
12 R. Lewinsohn, V. Glover and M. Sandler, Biochem. Pharmacol., 29 (1980) 777.
13 P.T. Kissinger, C.S. Bruntlett and R.E. Shoup, Life Sci., 28 (1981) 455.
14 R.D. Kobes, R.J. Wyatt and L.M. Neckers, J. Pharmacol. Methods, 3 (1980) 305.
15 C.J. Fowler, L. Oreland, J. Marcusson and B. Winblad, Naunyn-Schmiedeberg's Arch. Pharmacol., 311 (1980) 263.
16 K. Kobayashi, S. Kawai, T. Nakana and T. Nagatsu, J. Chromatogr., 274 (1983) 313.
17 A. Uji, S. Kawai and T. Nagatsu, J. Chromatogr., 221 (1980) 155.