Journal of Chromatography, 223 (1981) 188—192
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 782

Note

Studies on dopamine-converting enzymes in human plasma

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(First received August 13th, 1980; revised manuscript received October 17th, 1980)

The enzyme responsible for the biosynthesis of norepinephrine from dopamine, dopamine-β-hydroxylase (EC 1.14.17.1), has thus far been assayed by different procedures with a varying degree of complexity. One main approach is based on an enzymatic radiolabeling of the product using S-adenosyl-L-[methyl-^{14}C]methionine and a methyl transferase [1, 2]; another makes use of enzymatically liberated tritiated water from [7-$^{3}H_2$]dopamine [3]. In a third type of procedure, derivatization to form a fluorescent product, combined with thin-layer or liquid column chromatography and spectrofluorimetric detection, is utilized [4—7]. Recently a report describing an assay method based on the use of reversed-phase chromatography and electrochemical detection has appeared [8].

Our work, which was initiated before the latter study appeared, makes use of the same analytical principle. A quite different treatment of the reaction mixture prior to the chromatographic step was used, however. In our procedure no extraction on to aluminium oxide, a step which limits the information regarding product formation, was performed. With the technique described in this paper it has been possible, for the first time, to identify and study competitive enzymatic conversions of dopamine, due to the activity of other plasma enzymes, under the conditions used for the assay of dopamine-β-hydroxylase.

EXPERIMENTAL

Materials

All amine standards used were obtained from the Sigma Chemical Co. (St. Louis, MO, U.S.A.) as were dopamine-β-hydroxylase and pargyline. Catalase was obtained from Serva (Heidelberg, G.F.R.); all other chemicals for the assay were from Merck (Darmstadt, G.F.R.).

0378-4347/81/0000—0000/$02.50

A strong cation exchanger, Nucleosil SA 5 μm from Macherey, Nagel & Co. (Düren, G.F.R.), was used for the preparation of the analytical high-performance liquid chromatographic column.

Human plasma, obtained after blood sampling into heparinized tubes and centrifugation, was stored at —20°C if not used immediately.

Chromatographic procedure

The instrumentation and the chromatographic conditions used were the same as described previously [9] with the exceptions that a 170 × 4.6 mm analytical column was used and the detector cell was operated at an anode potential of +0.65 V (vs. Ag/AgCl) throughout.

Assay conditions

The reaction conditions were similar to those described by Wilcox and Beaven [3]. Addition of the plasma (1 ml) to the reaction medium (1.1 ml) which was also pre-thermostated to 40°C was taken as zero time. At different times a 100-μl aliquot was removed and mixed immediately with 250 μl of 0.8 *M* perchloric acid. The mixture was then centrifuged for 15 min at 9000 *g* to remove any protein precipitate. The clear supernatant was taken directly for analysis. The injected volume (20 μl) corresponded to 2.7 nmol of dopamine at 0% conversion and accordingly to 27 pmol of norepinephrine after 1% conversion to this product.

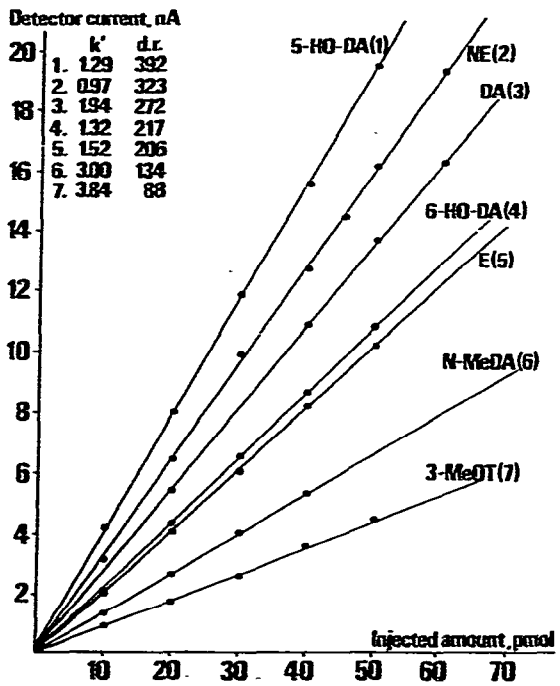

Fig. 1. Concentration dependence of the detector response for the various amine standards. Abbreviations: (1) 5-HO-DA = 5-hydroxydopamine; (2) NE = norepinephrine; (3) DA = dopamine; (4) 6-HO-DA = 6-hydroxydopamine; (5) E = epinephrine; (6) N-MeDA = N-methyldopamine; (7) 3-MeOT = 3-methoxytyramine.

Identification of reaction products

Identification of the reaction products was effected by rechromatography of the sample after the addition of an amine standard and observation of eventual peak superimposition. For an unequivocal identification, peak coincidence was verified in a different chromatographic system by the use of a reversed-phase analytical column.

RESULTS AND DISCUSSION

The evaluation of the chromatograms obtained from serial dilutions of a mixture of amine standards is summarized in Fig. 1. It shows the linear detector response for the compounds investigated, their capacity factors (k') and their detector response values (d.r.) obtained in the system expressed in pA/pmol at maximum peak height. The detection limit (signal-to-noise ratio = 2) can be as low as 0.1 pmol for norepinephrine under optimal conditions, which means that with the assay method described here as little as 0.02% conversion of dopamine can be measured, i.e. the true initial velocity can easily be computed.

In Fig. 2 typical chromatograms, illustrating the dopamine metabolites formed after a certain reaction time under the condition of the assay and ob-

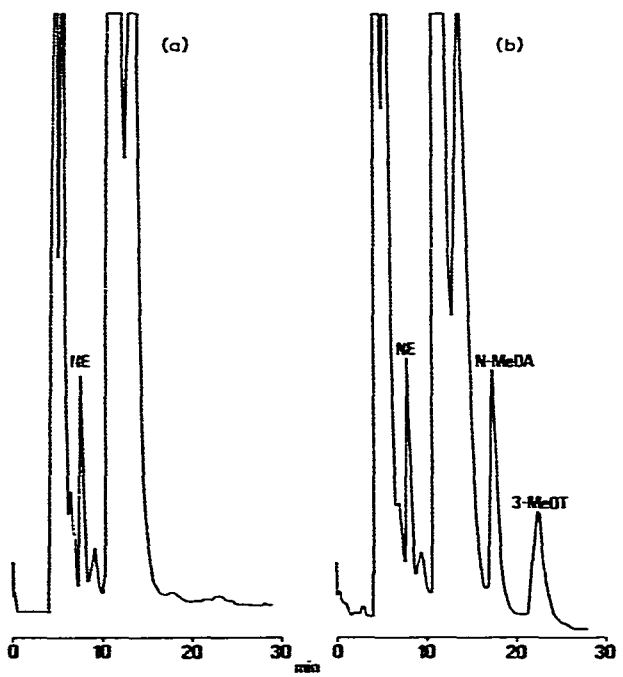

Fig. 2. Chromatograms showing the product composition (for abbreviations see Fig. 1) after a reaction time of 60 min. (a) Commercial enzyme preparation; (b) plasma sample. Buffer: 1 *M* sodium acetate (pH 5.2). Substrate concentration: 0.476 m*M*. The concentrations of all other reagents were as described in ref. 3. Pargyline was used as the sole monoamine oxidase inhibitor.

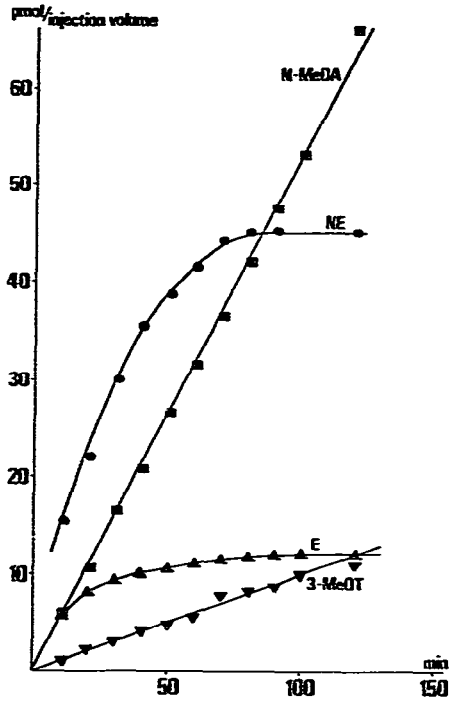

Fig. 3. Plasma-induced dopamine metabolite formation as a function of time. For reaction conditions see legend to Fig. 2. For abbreviations see Fig. 1.

tained with a commercial enzyme preparation (Fig. 2a) and with plasma as enzyme source (Fig. 2b), are shown. The chromatograms exhibited some peaks corresponding to electroactive species whose identity could not be established. The appearance of the identified metabolites as a function of time is demonstrated by Fig. 3. It is noteworthy that the formation of N-methyldopamine is completely linear with time, whereas norepinephrine formation is not. Similarly, 3-methoxytyramine increases linearly, while epinephrine appears soon to reach a steady-state concentration.

Blank experiments, carried out with boiled, centrifuged plasma, gave chromatograms showing the dopamine substrate as the only observable electroactive component.

CONCLUSION

Compared to other methods used for the determination of dopamine-converting enzymes such as dopamine-β-hydroxylase in biological samples, the method described here, in which electrochemical detection combined with high-performance liquid chromatography is utilized, appears to be superior for a variety of reasons. First, the extremely high sensitivity of the detector that can be achieved makes it possible to detect very low enzyme concentrations. Secondly, the ability of the detector to respond to a wide variety of compounds possessing an electro-oxidizable structural element (i.e. here the 3,4-dihydroxyphenyl or 3-methoxy-4-hydroxyphenyl moiety) means that metabolite formation due to the action of different enzymes can con-

veniently be studied kinetically as competitive reactions. Finally, the method does not rely on derivatization or extraction procedures, which obviates the need to use internal standards and calculations with respect to derivatization yields or recoveries.

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