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PRELIMINARY COMMUNICATIONS

EVIDENCE FOR AN ENZYMATIC p-TYRAMINE-DEHYDROXYLATING SYSTEM IN RABBIT BRAIN PREPARATIONS IN VITRO

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Evidence for the ring dehydroxylation in vivo of biogenic amines has been presented by a number of investigators [1-6]. However, in mammals, this type of reaction appears to be limited to a small number of substances, and even in these cases to be of little quantitative significance [5].

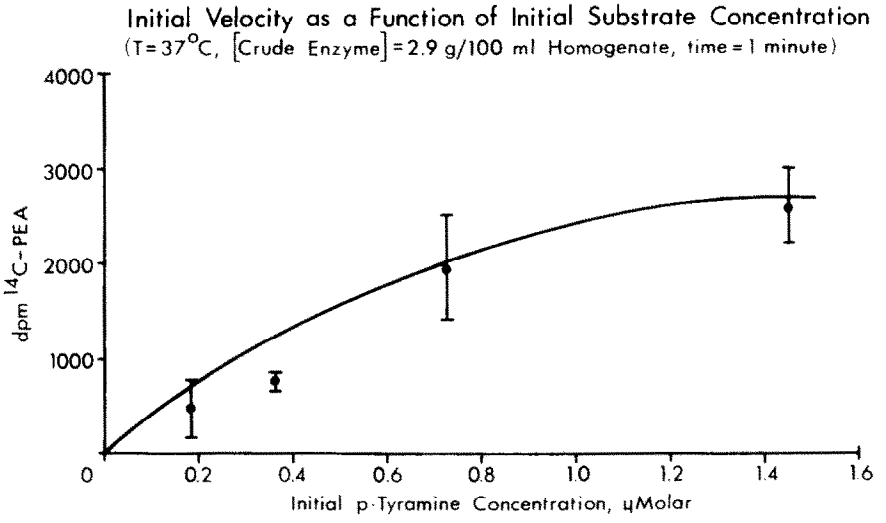
The conversion of p-tyramine (TRM) to 2-phenylethylamine (PEA) has been substantiated in the central nervous system after intraventricular injection of labeled TRM [3,6]. The importance of elucidating the nature and factors governing this reaction is apparent because it completes a pathway linking dopamine and PEA. The existence of this metabolic route has already been partly established by recent work showing the brain conversion of dopamine to TRM in vivo [1]. This biochemical route could prove of importance in the pathophysiology of certain diseases involving alteration of dopamine mechanisms [3,6]. In this study, we present some preliminary data supporting the enzymatic nature of this conversion of TRM to PEA, and we also describe an experimental system for the further characterization of this reaction.

The radiolabeled amines, PEA (custom-made ring [^{3}H]-PEA, sp. act. 2.0 Ci/m-mole) and TRM ([1-^{14}C]-TRM, sp. act. 12.4 mCi/m-mole, and [^{3}H]-TRM (G)·HCl, sp. act. 9.75 Ci/m-mole), were obtained from Amersham Searle and New England Nuclear. Before use, their purity (>95 per cent) was examined by thin-layer chromatography (t.l.c.) [Quanta Q_2 alumina-oxide glass plates; ter-amyl alcohol-20% aqueous methylamine (4:1), 6-8 hr at 32°], followed by t.l.c. radioactive scanning analysis [3,6] (Packard model-7200 radio-chromatogram scanner). All other chemicals and solvents were of the finest grade commercially available and were used without further purification.

White male New Zealand rabbits (2.0 to 2.5 kg, 9 to 11-weeks-old) were used as experimental animals. After decapitation, the brains were immediately removed, the main surface blood vessels and blood clots were carefully dissected out, and the tissue was quickly rinsed in chilled saline (0.9% NaCl), patted dry, and weighed (~ 8 g). The whole brains (two per

experiment) were homogenized in a 0.1 M phosphate buffer [1:2.5 (w:v), pH 7.4, 2.5% in Triton X-100], centrifuged (10,000 g, 10 min), and the supernatant was used as a crude enzyme preparation. These procedures were carried out at 0-4°.

Figure 1



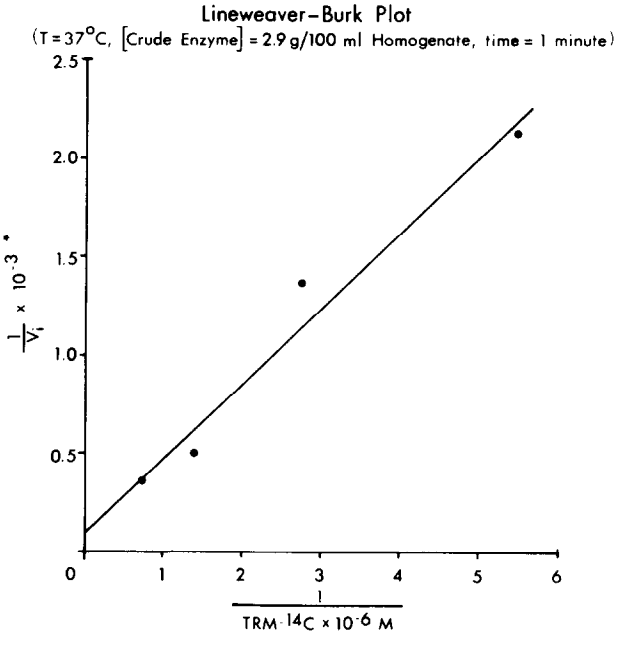
The reaction mixture containing the enzyme preparation and the monoamine oxidase inhibitor pargyline (final concentration range of 0.3 to 24 per cent and 2 X 10^{-5} M, respectively) was completed to a volume of 4.75 ml with buffer phosphate and incubated for 10 min (37°), prior to the addition of different TRM [1-^{14}C] concentrations (in 0.25 ml phosphate buffer; approximately 8 X 10^5 dis./min) (Fig. 1). PEA was then extracted from samples (1.2 ml), taken at different time intervals, as previously described [7]. [^{3}H]-TRM (0.08 ng, ~ 1 X 10^4 dis./min) was added to one third of the sample (which was used to estimate the specificity of the PEA separation procedure); another third of the sample received [^{3}H]-PEA (0.36 ng, ~ 1 X 10^4 dis./min) as internal standard to determine the percentage of recovery of the newly synthesized PEA-[^{14}C]; the rest of the initial sample was processed without further additions. Half of the hexane PEA-containing fraction was evaporated down into 1 N HCl (room temperature) and the residue was dissolved in a 2,5-diphenyloxazole, [1,4-bis-2-(4-methyl-5-phenyloxazolyl) benzene], Triton X-100, toluene (5 g, 0.9 g, 250 ml, 750 ml) counting mixture and used for PEA-[^{14}C] estimation (differential double isotope counting). Further confirmation of the presence of PEA-[^{14}C] was obtained by subjecting the rest of the samples to

t.l.c. followed by t.l.c. radioscanning (for details, see Refs. 3 and 5). The area corresponding to labeled PEA was scraped off the plate and counted. Blanks, using boiled homogenates plus ^{14}C-labeled TRM, were carried out throughout the entire experimental procedure, and the dis./min obtained were subtracted from the radioactivity present in the actual experimental samples. Using this procedure, we have efficiently separated newly synthesized labeled PEA from its radioactive precursor TRM, as well as from other possible interfering metabolites [3,5,6].

There was no statistically significant activity of either ^{14}C (blanks) or ^{3}H (sample aliquot with added [^{3}H]-TRM) in the corresponding n-hexane PEA-containing fraction. Furthermore, and considering that the radioscanning system has a 15-20 per cent activity-recording efficiency for ^{14}C and 0.1 to 1.0 per cent for ^{3}H, t.l.c. of this fraction (actual experiments) showed only one peak corresponding to ^{14}C-labeled PEA, whereas no radioactivity was detected from the blanks.

Figure 1 represents the initial velocity (1 min) for the p-dehydroxylation reaction of TRM to PEA. This graph, together with the Lineweaver-Burk plot of the same data (Fig. 2), shows the existence of a linear relationship between initial TRM concentration^{-1} (range, 0.2 to 1.6 μM) and product (PEA) formation^{-1}.

Figure 2



*V_i expressed as dpm ^{14}C-PEA formed per minute

The rather large standard error of the PEA values (Fig. 1) is probably due to the relative heterogeneity of the crude enzyme preparations used. Each point represents the average

± S.E. of five to seven experiments done in duplicate. Results are corrected for PEA recovery from brain incubates (range, 35-42 per cent).

It is clear from the present experiments that crude (10,000 g supernatant) rabbit brain preparations can convert significant amounts of TRM to PEA at all enzyme concentrations tested (0.3 to 24.0 per cent). Furthermore, our results, although of only a preliminary nature, show a linear relationship between the reciprocal of substrate concentration and initial reaction rate. These findings support the existence of a brain TRM-dehydroxylating enzymatic system and provide further evidence for a central nervous system metabolic pathway linking dopamine to PEA [3,6].

At present, there is no satisfactory evidence as to the possible physiological or pharmacological role of such a biochemical route, although given the short half-life [8,9] of brain PEA (which is both rapidly metabolized by MAO_B [10] and could readily leave the brain [11]), this pathway could be involved in a buffer-like mechanism regulating excessive accumulation of dopamine or TRM.

Undoubtedly, a better characterization of this new enzymatic reaction will have to be accomplished in order to understand its possible biological role.

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