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RAPID COMMUNICATION

DISCOVERY OF A NOVEL SOLUBLE FORM OF MONOAMINE OXIDASE IN THE RAT BRAIN

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We encountered a novel soluble form of monoamine oxidase (MAO) while studying dopamine metabolism in the arcuate nucleus of the rat brain. The presence of this isozyme in the extracellular space challenges the current concept that centrally released dopamine and other catecholamines undergo reuptake and partial deamination by mitochondrial MAO and are eventually salvaged into vesicles (1).

MATERIALS AND METHODS

Ten- to twelve-week-old male Sprague-Dawley rats (260-280 g) were anaesthetized with inactin (100 mg/kg, i.p.). A microdialysis probe (Cat. No. 8309551, Carnegie Medicin) was implanted into the arcuate nucleus/median eminence of each animal at the following coordinates with reference to the bregma: A-P 2.8 mm, ML 0.25 mm; depth from skull surface = 10.35 mm. Ringer solution containing 38 μM radiolabelled [7-^{14}C]dopamine (Amersham, 56 mCi/mmol) was perfused through the probe at a rate of 0.5 μL/min using a microinjector (CMA 100, Carnegie Medicin). Perfusate (7.5–μL aliquots) was collected in a refrigerated fraction collector (CMA 200, Carnegie Medicin). Radiolabelled dopamine and its metabolite in the perfusate were then separated by high performance liquid chromatography and the radioactivity was quantitated by a coupled radioisotope detector as described previously (2, 3). Briefly, the chromatography system consisted of a Waters 510 solvent delivery pump, a Rheoldyne 7125 injector, a Merck 5-μm C_{18} column, and a Beckman radioisotope detector (model 710). The mobile phase was a mixture of 0.1 M citric acid, 0.1 M sodium acetate, 0.1 mM sodium octyl sulfate, 0.15 mM ethylenediaminetetraacetate, 0.2 mM dibutylamine, and 10% (v/v) methanol, pH 4.1. The flow was isocratic at a rate of 1 mL/min. The efficiency of the isotope detector was 95% (also see legend of Fig. 1 for further chromatographic details). The chromatographic elution profile of DOPAC and the O-methylated primary and secondary metabolites of dopamine, namely 3-O-methyldopamine and 3-methoxy-4-hydroxyphenylacetic acid (HVA), respectively, were also determined using the same chromatographic separation method but with electrochemical detection (instead of radioisotope detection) as described in detail previously (4). In addition, as radiolabelled DOPAC is not commercially available, the elution profile of radiolabelled DOPAC generated by the brain dialysate was verified with that generated by purchased semi-purified MAO (Sigma) as described previously (2).

The *in vitro* recovery of each probe (defined as the ratio of dopamine concentration in the perfusate to that in the non-perfused Ringer solution) was determined before implantation. The average *in vitro*

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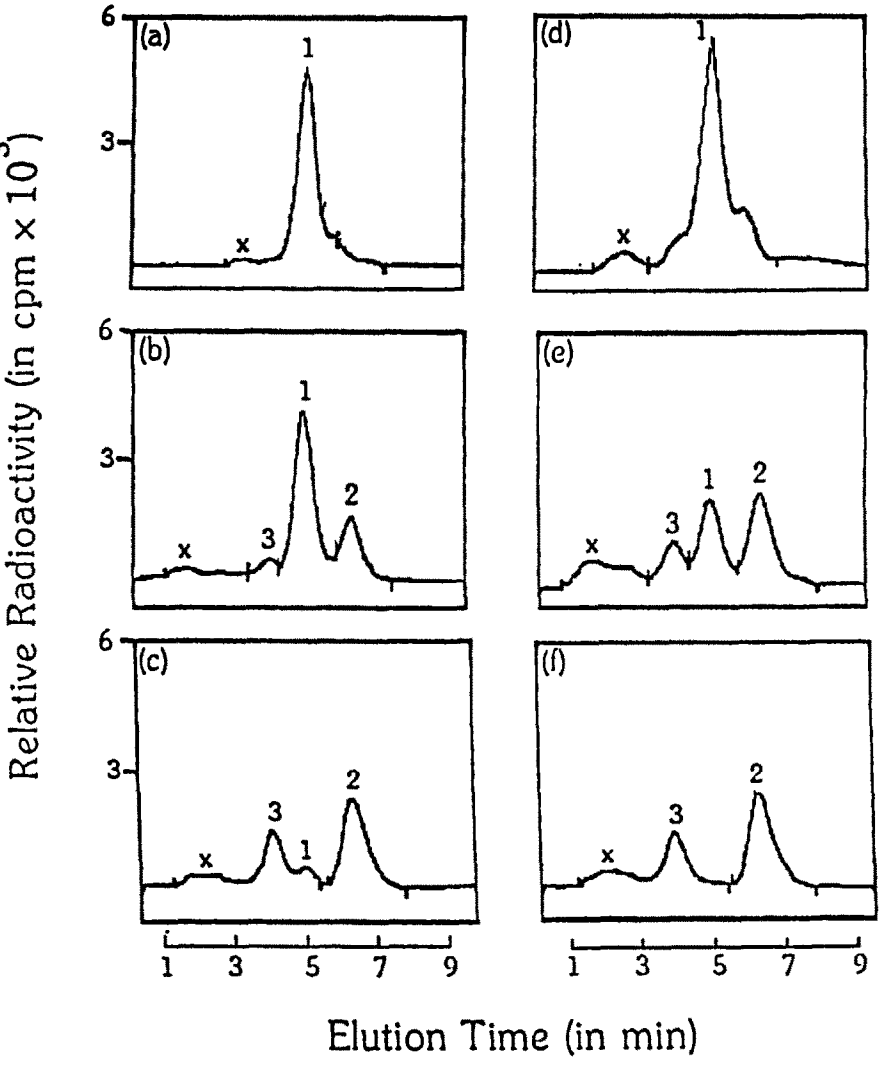

Fig. 1. Chromatograms showing the deamination of radiolabelled dopamine (peak 1) to DOPAC (peak 2) by a soluble isozyme of brain MAO. Peak 3 and X are unidentified compounds. Left chromatograms: (a) control chromatogram of a 5-μL sample of Ringer solution containing 38 μM radiolabelled dopamine (56 mCi/mmol); (b and c) chromatograms of 5-μL samples of the 2nd and 11th aliquots, respectively, of the same Ringer solution after perfusion through the dialysing probe. The average total radioactivity present in the three 5-μL samples of the perfusate was 21,250 $\pm$ 1,030 cpm and the radioactivities of peaks 3, 1 and 2 (in term of %) were 12, 60, and 22, respectively, for chromatogram (b) and 25, 12, and, 51, respectively, for chromatogram (c). Right chromatograms: each chromatogram was obtained from a 5-μL sample of a mixture containing 15 μL of perfused Ringer solution and 8 μL of a Ringer solution containing 145 μM radiolabelled dopamine (65 mCi/mmol) immediately after mixing (d), 50 min after mixing (e), and 100 min after mixing (f). The average total radioactivity present in the three 5-μL samples of the mixture was 28,900 $\pm$ 1,300 cpm and the radioactivities of peaks 3, 1 and 2 (in term of %) were 17, 31, and 44, respectively, for chromatogram (e) and 26, 0, and 57, respectively, for chromatogram (f). The unidentified peaks did not belong to the O-methylated metabolites of dopamine. The retention times of dopamine, DOPAC, 3-O-methyldopamine and HVA were 5.2 $\pm$ 0.2, 6.3 $\pm$ 0.2, 11.5 $\pm$ 0.2, and 19.5 $\pm$ 0.3 min, respectively.

recovery was 78 ± 9%, indicating that at least 78 ± 9% of the labelled dopamine should remain in the perfusate. At the end of each experiment, the correct location of the probe was verified histologically as described previously (5).

RESULTS AND DISCUSSION

The left chromatograms of Fig. 1 show that the perfused radiolabelled dopamine was metabolised to dihydoxyphenylacetic acid (DOPAC). However, the extent of the metabolism was, in most cases, much greater than 78%, i.e. greater than the *in vitro* recovery of the probe. One possible explanation was that a soluble MAO isozyme had been dialysed into the perfusate which continuously hydrolysed dopamine to DOPAC. The following experiment was designed to investigate this possibility.

Ringer solution without radiolabelled dopamine was similarly perfused and collected. Four microliters of Ringer solution containing 145 μM radiolabelled [7-^{14}C]dopamine (56 mCi/mmol) was added to each 7.5 μL of collected perfusate. Five microliters of this mixture was immediately injected into the HPLC system and another 5 μL after 30 min of standing in the dark at ambient room temperature (25^{0}). Similar incubations were carried out with other aliquots of perfusate and the mixtures analyzed after various periods of standing (up to 100 min). The results showed that labelled dopamine was metabolised continuously to DOPAC. Control samples containing non-perfused Ringer and similar portions of labelled dopamine showed no dopamine hydrolysis during the same period of standing (up to 100 min). The right chromatograms of Fig. 1 show the time-dependent metabolism of labelled dopamine to DOPAC. For inhibition study, pooled perfusate was aliquoted into five 4-μL samples. Two μL of Ringer solution was added to the first sample and 2 μL of various concentrations (4 μM to 4 mM) of clorgyline was added to the remaining four samples, respectively. The enzyme action was started by adding 2 μL of Ringer solution containing 145 μM radiolabelled [7-^{14}C]dopamine (56 mCi/mmol). The formation of DOPAC in the samples was concentration-dependently inhibited by clorgyline; complete inhibition occurred at a 1 mM concentration of the inhibitor. Similar study with deprenyl showed that it was a much weaker inhibitor with partial inhibition (25-32%) occurring at 1-10 mM. The enzyme also metabolised noradrenaline and tyramine in a time-dependent manner, but at a slower rate than dopamine (with tyramine being the poorer substrate of the two). Because MAO-A as well as MAO-B obtained from different tissues and animal species are known to deaminate the same substrates at different rates (6), it is difficult to base the identity of the two forms of MAO on substrate specificity. However, the complete inhibition of the enzyme by 1 mM clorgyline and partial inhibition by up to 10 mM deprenyl indicate that it is likely to be MAO-A (6, 7).

As the probe has a molecular cut-off of 20,000 Da, the molecular weight of the isozyme is probably less than 20,000. Because perfusate obtained with some probes contained little or no MAO isozyme, it is possible that the molecular weight of the isozyme borders on the upper limit of the molecular cut-off and that some probes had a smaller cut-off than others. This would rule out the possibility of the enzyme being the known soluble plasma amine oxidase as the latter has a molecular weight in excess of 195,000 (8, 9). The likely extracellular location of the isozyme is supported by the fact that microdialysis probes cause minimum damage to nervous tissue (10). Furthermore, the isozyme was detectable 28 hr after implantation when most neurons had regained physiological functions (11).

Although soluble forms of MAO have been reported to be present in the rat brain (12), our findings show for the first time the presence of a soluble form of MAO in the extracellular fluid of the brain. More importantly, they may indicate that extracellular deamination of catecholamines is an important, hitherto unknown, physiological process of termination in certain regions of the brain.

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