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Short communication

Effect of 6-fluoro-m-tyrosine on dopamine release and metabolism in rat striatum using in vivo microdialysis

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Abstract

6-[^{18}F]Fluoro-m-tyrosine (FMT) is a positron emission tomography (PET) imaging agent for the aromatic L-amino acid decarboxylase enzyme. Its parent compound, L-m-tyrosine (LMT) induces behavioral effects in rodents via dopamine release. To assess the potential pharmacologic effect of FMT, its role in dopamine release and metabolism in rat striatum was compared with LMT and L-DOPA using in vivo microdialysis. Results indicate that FMT will not have the same dopamine-induced behavioral effects as LMT.

Theme: Neurotransmitters, modulators, transporters and receptors

Topic: Catecholamines

Keywords: 6-Fluoro-m-tyrosine; In vivo microdialysis; Dopamine release

L-m-Tyrosine (LMT) is a monohydroxy analog of 3, 4-dihydroxyphenylalanine (L-DOPA) which initially drew interest as a therapeutic agent for Parkinson's disease (PD) because of its anti-reserpine activity. The pharmacologic actions of LMT included (1) an awakening effect on reserpinized mice [2], (2) protective effects on reserpine-induced depletion of catecholamine stores [5], (3) reversal of reserpine-induced suppression of the conditioned avoidance response [14] and (4) other behavioral effects similar to that of L-DOPA action in animal models of PD [1,24]. Unfortunately, clinical trials in PD patients proved m-tyrosine ineffective in ameliorating the symptoms associated with parkinsonism [6,20].

Nevertheless, LMT is an excellent substrate of the aromatic L-amino acid decarboxylase (AAAD) enzyme [8] and its behavioral and pharmacologic effects in rodents have been shown to result from the action of its AAAD product, m-tyramine (MTA) [12,21]. A possible mechanism proposed for the biological activity of MTA involves an indirect agonist effect whereby MTA is thought to enter storage vesicles inducing the displacement and release of stored dopamine into the synapse.

Recently, a fluorinated m-tyrosine analog, 6-fluoro-m-tyrosine (FMT), was suggested and developed as an imaging agent to assess monoamine systems using positron emission tomography (PET) [7,10]. FMT is attractive as a PET radiotracer because of its shared biochemistry with LMT including brain uptake via the large neutral amino acid (LNAA) transporter [9], high affinity for AAAD as substrate [8], and poor affinity for the dopamine-deactivating enzyme catechol-O-methyl-transferase (COMT) due to its non-catechol structure. More recent studies support the utility of FMT as a selective PET tracer to assess AAAD activity [4,11].

In order to further characterize the pharmacology of FMT, we performed in vivo microdialysis studies to monitor the effect of systemically administered FMT on the concentration of dopamine and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), in the extracellular space in the striatum of anesthetized rats. By comparing the effect of FMT on dopamine release and metabolism in rodents with those of better characterized analog drugs, LMT and L-DOPA, the

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potential stimulant effect of FMT in human subjects can be evaluated.

L-DOPA, DL-m-tyrosine, and α-chymotrypsin were obtained from Sigma Chemicals (St. Louis, MO) while DOPAC, DA, and HVA were obtained from Aldrich Chem. Co. (Milwaukee, WI). LMT was resolved and separated from the racemic commercial DL-m-tyrosine using the method of Tong et al. [23]. DL-FMT was prepared by a synthetic scheme adapted from the method of Snyder et al. [22]. The purified product was analyzed by high resolution mass spectrometry and multinuclear (^{1}H, ^{13}C, ^{19}F) NMR spectroscopy and shown to be the desired compound. The L-FMT stereoisomer was similarly isolated from the racemic product using the method of Tong et al. [23]. The experiments in this study were done using the active levo stereoisomers of FMT, LMT, and L-DOPA.

Following an experimental protocol approved by the University of Wisconsin Animal Care and Use Committee, microdialysis probes (CMA Microdialysis, Solna, Sweden) were placed, using a Kopf stereotaxic frame, into the left striatum of 250–400 g male HSD rats (Harlan Sprague Dawley, Madison, WI) anesthetized with urethane (1.5 g/kg, i.p.). The coordinates used for probe placement were AP: bregma +0.5 mm, L: 2.5 mm, DV: dura −7 mm based on the rat atlas of Paxinos and Watson [19]. Artificial CSF (flow-rate=2 μl/min) was perfused through the probe and 20-min samples (40 μl) were collected in tubes containing 20 μl 1 N perchloric acid. The microdialysis probes were calibrated in vitro using a mixture of 1 μM each of DOPAC, DA, and HVA to determine efficiency. Only probes with efficiencies >20% were used in these studies. Samples were directly analyzed using an HPLC system consisting of a reversed phase C18 column (3 μ, 100×4.6 mm) (Alltech Associates, Deerfield, IL), ion pairing mobile phase and BAS LC-4C electrochemical detector (Bioanalytical Systems, West Lafayette, IN) set at +0.8 V. Peak areas were calculated and used as the measure of DOPAC, DA, and HVA concentration. Basal levels of DOPAC, DA, and HVA in each study were determined as the means of at least three microdialysate samples taken prior to the injection of the drugs-L-DOPA (60 mg/kg), L-m-tyrosine (LMT) (60 mg/kg) and FMT (60 and 90 mg/kg).

The time-courses of extracellular concentrations of DOPAC, dopamine, and HVA as expressed as percentage of their respective basal concentrations following the administration of L-DOPA, LMT, and FMT are shown in Fig. 1. The error bars are S.E.Ms obtained from two to four animals per drug treatment. When given without a peripheral AAAD inhibitor, the increase in extracellullar DA after acute L-DOPA (50 mg/kg, i.p.) has been reported to be transient (occurring within 20 min postinjection) [3]. Although we did not observe this short-lived increase in DA in our study, we did find continually increasing

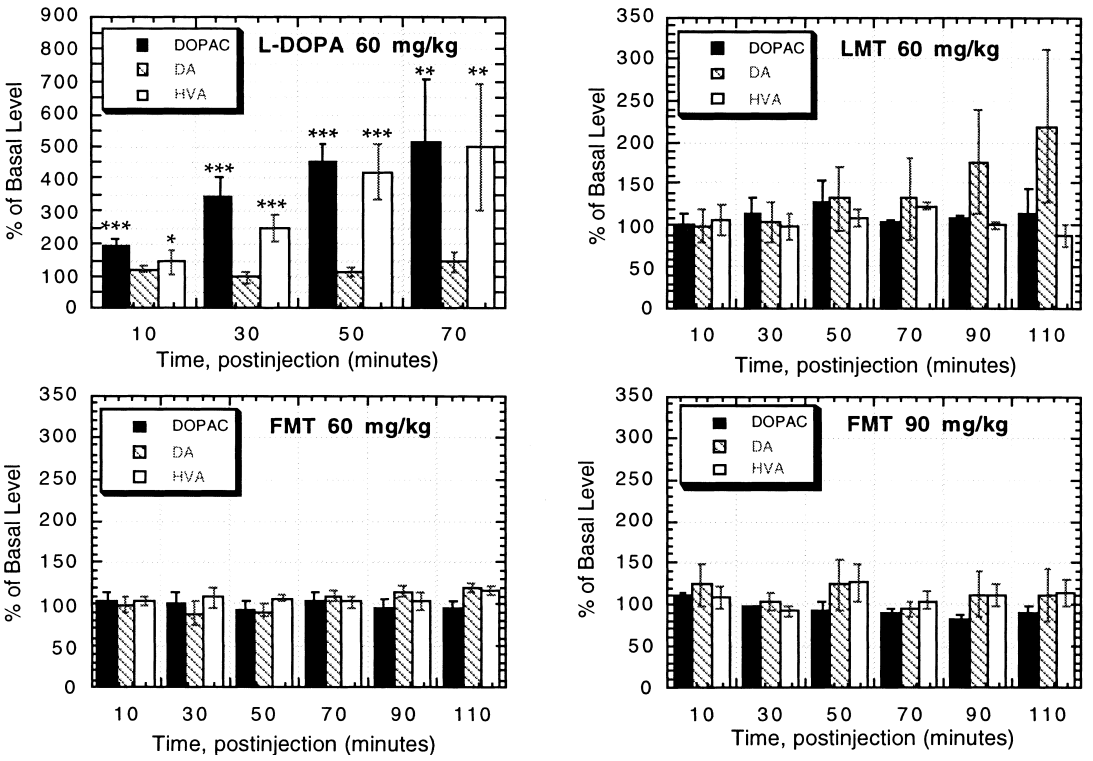

Fig. 1. The time-course of extracellular DOPAC, DA, and HVA in the rat striatum following the i.p. injection of L-DOPA (60 mg/kg), LMT (60 mg/kg), and FMT (60 and 90 mg/kg). The effect of each treatment is shown in their respective panels. Each value is expressed as the percentage of the basal level of each compound and is the mean obtained in two to four rats and the error bars refer to S.E.M. Those values without asterisks are not significantly different from basal levels while those with asterisks have the following *t*-test significance: $*P<0.05$, $**P<0.01$ and $***P<0.001$.

extracellular concentrations of DA metabolites, DOPAC and HVA, similar to that observed by Brannan et al. [3]. Since both metabolites are derived from dopamine, it is reasonable to assume that the observed increase in DOPAC+HVA levels correspond to the increased formation of DA originating from the exogenous L-DOPA and its rapid metabolism. Thus, in the L-DOPA panel in Fig. 1, in vivo microdialysis results show that 70 min after 60 mg/kg L-DOPA i.p. injection, there is a 10-fold increase in extracellular dopamine. In comparison, an equal dose of FMT caused no change in DA, DOPAC, and HVA levels at 70 min, 90 min and 110 min (Fig. 1). Similarly, no change was observed at a higher FMT dose of 90 mg/kg (Fig. 1). In contrast to both L-DOPA and FMT, LMT (60 mg/kg) was found to increase DA in the extracellular space greater than two-fold 110 min postinjection, while DOPAC and HVA levels were unchanged at all times. However, this increase in dopamine levels did not reach significance ($P>0.05$).

The increases in DOPAC and HVA found after L-DOPA administration are likely due to the inability of vesicles to store the relatively excessive amounts of DA produced from exogenous L-DOPA. Although LMT-induced DA increase in extracellular space observed in this study was not statistically significant, the effect on DA tissue levels was not determined. A study by Smyth et al. [21] using a higher LMT dose (150 mg/kg) observed tissue DA levels 60 min after i.p. injection to be reduced to about half that of basal level DA. This LMT-induced DA reduction can be blocked by AAAD inhibition in support of the role of MTA as the causative agent [21]. Furthermore, the suggestion by Smyth et al. [21] that MTA may displace DA is supported by previous findings that MTA is taken up by chromaffin granules [13,17]. The displaced DA is likely quickly metabolized since the systemic injection of m-tyrosine produced intense behavioral stimulation only when DA metabolism by MAO was blocked [12].

On the other hand, the results of this study show that DA and its metabolites in the extracellular space are not affected by the systemic injection of FMT up to an i.p. dose of 90 mg/kg. This finding is supported by our previous observation that decarboxylated FMT, FMTA, is poorly taken up by chromaffin granules compared to fluorodopamine and MTA [13]. In vivo studies previously demonstrated that after its administration, FMT is rapidly decarboxylated to form FMTA which, in turn, is rapidly oxidized by MAO as shown by the rapid formation, the dominance and the persistence of the MAO product of FMTA, fluoro-hydroxyphenylacetic acid, in both extracellular space [16] and whole striatal tissues in rodents [18] and in non-human primates [15]. The rapid MAO oxidation of FMTA is consistent with its lack of vesicular uptake and protection [13]. Thus, unlike LMT, even with MAO inhibition, FMT would not be expected to have dopamine-induced behavioral effects at any dose.

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