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Research Report

Aromatic L-amino acid decarboxylase turnover in vivo in rhesus macaque striatum: A microPET study

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Abstract

The aromatic L-amino acid decarboxylase (AAAD) is involved in the de novo synthesis of dopamine, a neurotransmitter crucial in cognitive, neurobehavioral and motor functions. The goal of this study was to assess the in vivo turnover rate of AAAD enzyme protein in the rhesus macaque striatum by monitoring, using microPET imaging with the tracer [^{18}F]fluoro-*m*-tyrosine (FMT), the recovery of enzyme activity after suicide inhibition. Results showed the AAAD turnover half-life to be about 86 h while total recovery was estimated to be 16 days after complete inhibition. Despite this relatively slow AAAD recovery, the animals displayed normal movement and behavior within 24 h. Based on the PET results, at 24 h, the animals have recovered about 20% of normal AAAD function. These findings show that normal movement and behavior do not depend on complete recovery of AAAD function but likely on pre-synaptic and post-synaptic compensatory mechanisms.

Theme: Neurotransmitters, modulators, transporters and receptors
Topic: Monoamine synthesis

Keywords: Aromatic L-amino acid decarboxylase; Protein turnover; MicroPET imaging; Parkinson's disease

1. Introduction

The aromatic L-amino acid decarboxylase (AAAD) (EC 4.1.1.28) is the second enzyme in the biosynthetic pathway to important monoamine neurotransmitters—dopamine (DA), norepinephrine (NE) and serotonin (5-hydroxytryptamine or 5 HT). AAAD converts L-DOPA to DA which in turn is converted in NE neurons by dopamine β-hydroxylase (DβH) to NE while 5-hydroxytryptophan is converted by AAAD to 5 HT. Because the AAAD enzymic Km is higher than the normal levels of cerebral L-DOPA, conversion of L-DOPA to dopamine is rapid and has led many to believe that AAAD is not involved in dopamine regulation. However, there is now accumulating evidence that pre- and post-synaptic mechanisms alter AAAD activity via regulatory processes [6,29].

Parkinson's disease (PD) is a movement disorder characterized by a dramatic reduction in dopamine levels in the terminals due to degeneration of nigrostriatal dopamine neurons. Since its introduction in 1967 [7], dopamine replacement using L-DOPA has remained to be the most effective therapy to ameliorate PD symptoms. Thus, AAAD is thought to play the important role as rate-limiting step in L-DOPA therapy in PD. Because changes in AAAD activity have clinical consequences [21], better understanding of AAAD function is needed. One important aspect of AAAD function which needs elucidation is its turnover rate in vivo. In this study, AAAD turnover rate in normal non-human primate striatum was assessed in vivo by monitoring recovery of AAAD function after irreversible

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AAAD inhibition. This study involved the administration of a single dose of an AAAD suicide inhibitor, α-monofluoromethyl-DL-3,4-dihydroxyphenylalanine (FMDOPA) [14] followed by longitudinal assessment of striatal AAAD recovery by non-invasive microPET imaging using 6-[^{18}F]fluoro-*meta*-tyrosine (FMT), a selective imaging agent for estimating AAAD activity [8,9].

2. Materials and methods

2.1. Inhibitor synthesis and characterization

FMDOPA was synthesized de novo for this study following the method of Bey et al. [3] as we previously described in the synthesis of a close analog, α-monofluoromethyl-*m*-hydroxyphenylalanine (FM-*m*-tyrosine, FMFmT) [19]. After purification, FMDOPA was authenticated by ^{13}C- and ^{1}H NMR spectroscopy and validated by in vitro studies using rat kidney and striatal homogenates. Consistent with the results of Bey at al. [4], our in vitro results showed that FMDOPA has IC_{50} value comparable to those of other AAAD inhibitors–carbidopa, benserazide and NSD 1015–in inhibiting AAAD activity in these rat tissue homogenates (unpublished results).

2.2. Radiotracer synthesis

The PET tracer [^{18}F]FMT was prepared by the method of Namavari et al. [20]. Briefly, to achieve regioselective electrophilic radiofluorination, [^{18}F]F_2, produced as previously described [24], was bubbled into a fluorodichloromethane solution of a protected stannylated *m*-tyrosine derivative purchased from ABX Biochemicals (Radeberg, Germany). After removal of SnF_2 and other by-products using silica minicolumn, protective groups in the [^{18}F]-intermediate were removed by refluxing in 48% hydrobromic acid. The product [^{18}F]FMT was purified using a semi-preparative HPLC (Alltech C18 column, 10 μ, 250 × 10 mm) with a mobile phase which consisted of 0.02 M NaOAc pH 3.5 and made up with sufficient NaCl to be isotonic. The HPLC fraction containing the product was collected and sterilized by filtration through a 0.22 μ filter into a vented sterile vial prior to administration into the subjects.

2.3. Animal imaging: PET and MRI studies

Two male rhesus monkeys (designated as AT 97 and AU 18), both 7 years old and weighing 8 kg, were used in this study. The animals were anesthetized with ketamine (15 mg/kg IM) to allow administration DL-FMDOPA (25 mg/kg) into the peritoneum. Both animals were scanned serially at different times ranging from 4 h up to 8 days after FMDOPA injection. The animals were fasted overnight before each PET study. On the day of the PET study, anesthesia was initially induced with ketamine (15 mg/kg IM) to allow the transport of the animals to the Keck MicroPET Laboratory at the University of Wisconsin Waisman Center. Anesthesia during the scan was maintained with isoflurane (1%–2% in oxygen). The protocol used in these studies was approved by the UW Animal Care and Use Committee in compliance with NIH regulations on the use of non-human primates in research.

The microPET P4 scanner used in this study is a commercial dedicated small animal research scanner (22 cm bore) from Concorde Microsystems (Knoxville, TN). The calculated image resolution, using filtered back projection reconstruction, in 8 cm axial × 19 cm transaxial field of view (FOV), is 2 × 2 × 2 mm^3 with 2% sensitivity at center [27]. The anesthesized animal was positioned prone in the scanner bed with its head comfortably immobilized in a stereotactic holder (with ear, mouth and eye orbit bars) orienting the brain such that the PET slices paralleled the orbitomeatal (OM) line. Heart rate, SPO_2, respiratory rate and temperature were monitored throughout the scan and body temperature was controlled using a loose-fitting wrap blowing warm air around the animal (Bair Hugger, Arizant Healthcare, Eden Prairie, MN). A 15-min transmission scan using a ^{68}Ge point source was performed to verify proper positioning and for use in post-acquisition correction for photon attenuation. Data are recorded in list mode, permitting maximum post-analysis flexibility in time averaging. A 90-min dynamic 3D acquisition was begun simultaneously with the intravenous administration of 3.5–5.4 mCi [^{18}F]FMT. Each animal had previously undergone magnetic resonance imaging (MRI) on an Advantx 1.5 T instrument (General Electric Medical Systems, Waukesha, WI) under ketamine sedation. MR imaging included a spoiled gradient-echo (SPGR) sequence, a 3D acquisition reconstructed into contiguous 1.3-mm coronal slices providing good differentiation between gray matter and white matter.

2.4. PET image and region of interest analysis

After the scan, the list mode data were binned into sinograms with 20 frames (5 × 1 min, 5 × 2 min, 5 × 5 min and 5 × 10 min) and corrected for dead time and random coincidences. Images were then reconstructed with Concorde Microsystems proprietary software using OSEM, correcting for detector sensitivity and attenuation. The images were converted to Analyze format using an in-house imaging software program called SPAMALIZE [22]. Images were then smoothed with a 3 mm Gaussian filter in Spm99. The PET image was manually co-registered to each animal's anatomical and stereotaxic MRI T1 weighted image using SPAMALIZE and a region of interest (ROI) approach was used. Striatal and cerebellar regions were identified and drawn on the MRI T1 weighed images. The ROIs were then applied to the co-registered PET images for generating time activity curves (TACs) for the subsequent Patlak analysis [23]. The Patlak Ki values obtained were