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Mapping the amphetamine-evoked changes in [^{11}C]raclopride binding in living rat using small animal PET: Modulation by MAO-inhibition

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The performance of small animal PET for neuroreceptor studies in a psychopharmacological challenge paradigm is not yet well-described. Therefore, we used microPET and [^{11}C]raclopride to map the availability of dopamine $D_{2/3}$ receptors in brain of anesthetized rats, first in a baseline condition, and again after challenge with saline or D-amphetamine. Parametric maps of the specific binding (binding potential, *pB*) were calculated using a reference tissue input from cerebellum, and spatially normalized to a digitized stereotaxic coordinate system for rat brain. In volumes of interest (VOIs), the mean baseline *pB* ($n=6$) was 2.05 in dorsal striatum (caudate-putamen), and 1.34 in ventral striatum (nucleus accumbens), and did not significantly differ upon retest 2 h later. The availability of [^{11}C]raclopride binding sites at baseline was 8% higher in the right striatum. Challenge with amphetamine sulfate (1 mg/kg, i.v., $n=4$) decreased *pB* by 19% in both ventral and dorsal striatum. We have earlier predicted that blockade of monoamine oxidase (MAO) should potentiate the amphetamine-evoked dopamine release, thus enhancing the displacement of [^{11}C]raclopride binding *in vivo*. However, pretreatment of rats with pargyline hydrochloride (4 mg/kg, $n=4$; 20 mg/kg, $n=4$) 1 day prior to PET did not potentiate the amphetamine-evoked reduction in dopamine receptor availability within the extended striatum. We conclude that small animal PET can be used to investigate stimulant-induced dopamine release, but that the spatial resolution is insufficient to detect differences between relative changes in dorsal vs. ventral divisions of the rat striatum. Furthermore, the present results do not reveal potentiation of the amphetamine-evoked release of dopamine in rats with MAO inhibition.

Keywords: microPET; Raclopride; Amphetamine; Pargyline; Rats

Introduction

The release of intracellular pools of dopamine by amphetamine has been revealed in positron emission tomography (PET) studies with reversible ligands for dopamine $D_{2/3}$ receptors. In this paradigm, increased dopamine release is revealed by enhanced competition between interstitial dopamine and the benzamide antagonist [^{11}C]raclopride, for the same binding sites, resulting in reduced binding potential (*pB*, proportional to B_{max}/K_d). Amphetamine-evoked decreases in the binding of raclopride have been shown in several studies in rats *ex vivo* (Young et al., 1991; Cumming et al., 2002) and *in vivo* (Hume et al., 1992; Houston et al., 2004), cats (Ginovart et al., 2004), pigs (Lind et al., 2005), non-human primates (Alexander et al., 2005) and humans (Drevets et al., 2001; Cardenas et al., 2004). Microdialysis studies have shown the amphetamine-evoked release of dopamine in the rat ventral striatum to exceed that in the dorsal striatum (Di Chiara and Imperato, 1988); likewise the vulnerability of [^{11}C]raclopride binding to challenge with amphetamine (Lind et al., 2005; Drevets et al., 1999; Leyton et al., 2002) and nicotine (Cumming et al., 2003) has been reported to be greatest in the ventral striatum of living animals and humans. The high spatial resolution of microPET (2 mm) predicts that spatial heterogeneity in the vulnerability of [^{11}C]raclopride to competition from endogenous dopamine could likewise be detected within the extended striatum of living rats, but this has not hitherto been demonstrated.

Dopamine is degraded by both subtypes of the intracellular enzyme monoamine oxidase (MAO) (Azzaro et al., 1985). Inhibition of both MAO isozymes (MAO-A and MAO-B) with the suicide substrate pargyline increases the vesicular concentration of dopamine in rat striatum, and, to a lesser extent, also the cytoplasmic dopamine concentration (Buu, 1989). In rats, acute treatment with pargyline rapidly depletes the interstitial concentrations of acidic dopamine metabolites, while tending to increase the

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interstitial dopamine concentration by 50% (Cumming et al., 1992), although others report more substantial increases in interstitial dopamine (Javoy et al., 1973; Butcher et al., 1990; Vidal et al., 2005). The amphetamine-evoked dopamine release in rat striatal microdialysates was enhanced by pargyline pretreatment (Butcher et al., 1988) and also by the MAO-A selective inhibitor clorgyline (Butcher et al., 1990). Similarly, in guinea pigs, simultaneous blockade of MAO-A and MAO-B was without significant effect on basal dopamine efflux, but increased the tissue content of dopamine and enhanced the amphetamine-evoked dopamine release (Ilani et al., 2000), whereas the reversible MAO-A inhibitor harmine enhanced the impulse-dependent increase in interstitial dopamine (Iurlo et al., 2001). Based on these findings, we hypothesized that MAO inhibition would potentiate the amphetamine-evoked dopamine release in living striatum, to be revealed by a decrease in [^{11}C]raclopride *pB*. In an earlier study of Göttingen miniature pigs, MAO inhibition during 12 h was without discernible effect in the [^{11}C]raclopride-amphetamine paradigm (Jensen et al., 2006), possibly reflecting species differences in the regulation of monoamine metabolism.

In the present study, we first developed methods for the spatial normalization and averaging of parametric maps of [^{11}C]raclopride *pB* in brain of living rats. The test–retest stability of *pB* was determined during a 4 h long microPET scanning session. We then used a fine anatomical segmentation of the parametric maps to test for spatial heterogeneity in the vulnerability of [^{11}C]raclopride *pB* to amphetamine challenge in the rat. The specific activity of [^{11}C]raclopride was measured, and correlated with the magnitude of *pB* in order to test for mass-effects. Finally, we tested the hypothesis that pargyline treatment 1 day prior to the experiment should potentiate the effect of amphetamine in anesthetized rats: we compared the effects of low dose (4 mg/kg) and high dose (20 mg/kg) of pargyline on the [^{11}C]raclopride *pB* at baseline and after amphetamine challenge.

Methods

Chemicals

Acetonitrile and iodine were purchased from Bie and Berntsen, Denmark. Sodium phosphate monohydrate and DMSO were from Sigma-Aldrich, Denmark. Desmethyl raclopride and raclopride were generously donated by AstraZeneca R&D, Södertälje, Sweden.

Radiochemistry

[^{11}C]Raclopride was prepared by the method of Gillings (1999), with some modifications. [^{11}C]Carbon dioxide was prepared by the ^{14}N(p,a)^{11}C proton bombardment with the General Electric Medical Systems PETtrace 200 cyclotron, and converted to [^{11}C]methyl iodide using the GE MeI box. A 1 ml vial containing DMSO (400 µl), NaOH (7 µl) and *O*-desmethyl-raclopride (2.0 mg±0.2 mg) was sealed and the mixture was shaken vigorously until a dark green color appeared. The [^{11}C]methyl iodide (6–9 GBq) was then delivered in a stream of nitrogen via a needle inserted in the via. The nitrogen was vented through another needle, after deposition of the [^{11}C]methyl iodide in the reaction solution. The needles were then removed, and the mixture was heated at 90 °C for 5 min. Purification of [^{11}C]raclopride was performed by semi-preparative high-performance liquid chromatography (HPLC) (PerkinElmer model 200) equipped with a 5 ml injection loop. The mobile phase, consisting of 30% ethanol and 70% NaH_2PO_4 (70 mM), was delivered at a rate of 8 ml/min to a Suplex pKb-100 HPLC column (Sigma-Aldrich), under which conditions, the product retention time was 10–12 min. Product elution was monitored with serial on-line gamma detection of in-house design and UV–visible detection (Applied Biosystems model 759A, λ=230 nm). The main fractions containing product were collected, and the volume was reduced to approximately 1 ml under reduced pressure at 90 °C, and then formulated in isotonic saline. Determination of [^{11}C]raclopride specific activity was made using HPLC analysis with a Bondclone 10 C18 column, with a mobile phase consisting of 35% acetonitrile and 65% NaH_2PO_4 (70 mM, delivered at 3 ml/min). Mass concentration was calculated relative to pure raclopride standard with UV detection at 230 nm.

microPET scanning

Eighteen male inbred Lewis rats (Taconic, Denmark) weighing 277–317 g were used in the study, which was carried out according to the requirements of the Danish Experimental Animal Inspectorate. Until the day before the experiments, rats were group-housed and offered tapwater and feed *ad libitum* (Altromin 1324, Brogaarden, Denmark). The rats were assigned to four groups: test–retest (n=6), amphetamine challenge (n=4), and amphetamine challenge after MAO-inhibition (n=8). The latter rats were pretreated with subcutaneous pargyline hydrochloride (Sigma) at a mean dose of 4 (n=4) or 20 mg/kg (n=4) the evening before the PET study. The day of scanning, anesthesia was induced in a chamber filled with 5% isoflurane in a mixture of O_2 and air and maintained using a mask delivering isoflurane (1.8–2.0%) in O_2 (0.4 l/min) and air (1.5 l/min). Respiration frequency, rectal temperature and absence of tail and interdigital pain reflexes were motored during the entire procedure. Body temperature was maintained close to 36.5 °C using a heat lamp regulated by a rectal thermometer. Injection catheters were either inserted transcutaneously in the tail vein (n=7) or surgically in the femoral artery and vein (n=11). For the latter group, arterial blood samples (210 µl) were drawn two to three times during each double-scan session for analysis of blood gases and chemistry (ABL520, Radiometer, Copenhagen).

After surgery, rats were placed prostrate with their head in the aperture of the small animal tomograph (microPET R4, CTI Concorde), with the head held in place using a custom-built Plexiglas unit. The spatial resolution (FWHM) of the R4 tomograph is 2 mm at the centre of aperture (Knoess et al., 2003), indicating a volume resolution of 8 mm^3. At the beginning of each scanning session, an attenuation scan lasting 10 min was first performed using a ^{68}Ge point source. Dynamic emission recordings were initiated upon injection of [^{11}C]raclopride (9–41 MBq; mean 20 MBq). The 90 min long emission recordings consisted of 26 frames increasing in duration from 15 s to 10 min. At 2 h after the first tracer injection, a second [^{11}C]raclopride recording was initiated. All animals other than those in the test–retest group received D-amphetamine hydrochloride (1 mg/kg, i.v.) 30 min before the second tracer injection.

Immediately after completion of the second emission recording, rats were decapitated while still anesthetized. The brains were rapidly removed and frozen by immersion in a mixture of isopentane and dry ice or liquid nitrogen at −40 °C and stored at −80 °C until used for quantitative autoradiography, results of which are to be reported elsewhere.