ELSEVIER

Brain Research Bulletin 65 (2005) 1–9

BRAIN RESEARCH BULLETIN

www.elsevier.com/locate/brainresbull

Mapping the amphetamine-evoked dopamine release in the brain of the Göttingen minipig

Nanna Marie Lind[a,b,*], Aage Kristian Olsen[c], Anette Moustgaard[a,b], Svend Borup Jensen[c], Steen Jakobsen[c], Axel Kornerup Hansen[b], Sidse Marie Arnfred[a], Ralf Peter Hemmingsen[a], Albert Gjedde[c], Paul Cumming[c]

[a] *Department of Psychiatry, University Hospital of Copenhagen, Bispebjerg, Denmark*
[b] *Division of Laboratory Animal Science and Welfare, Department of Veterinary Pharmacology and Pathobiology, The Royal Veterinary and Agricultural University, 9 Ridebanevej, DK-1870 Frederiksberg C, Denmark*
[c] *PET Centre, Aarhus General Hospitals, Centre of Functionally Integrative Neuroscience, Aarhus, Denmark*

Received 10 August 2004; accepted 14 August 2004
Available online 25 December 2004

Abstract

The availability of dopamine $D_{2/3}$ binding sites in brain of six male and six female Göttingen minipigs was measured in a baseline condition and after challenge with amphetamine sulfate (1 mg/kg, i.v.) in PET studies with [^{11}C]raclopride. Maps of the binding potential *(*pB; B_{max}/K_d*)* of [^{11}C]raclopride were spatially normalized and co-registered to a common stereotaxic coordinate system for pig brain. The pB maps were then analyzed by volume of interest and voxel-wise comparisons of gender and condition. The mean baseline pB tended to be 10–20% higher in striatum of the female group, but this gender difference was not significant. Variance of the mean baseline pB was higher in the males (44%) than in females (30%), but there was no correlation between pB and individual plasma cortisol or testosterone concentrations. Using statistical parametric mapping, we detected a focus in the right posterior putamen where the magnitude of the amphetamine-evoked decrease in pB was greater in the male than in the female group. Thus, the spatial pattern of reactivity of dopamine $D_{2/3}$ receptor availability to amphetamine challenge is not identical in male and female pigs. Within the entire population, the decline in pB evoked by amphetamine (ΔpB) was greater in the ventral striatum (−28%) than in the caudate nucleus (−17%), consistent with earlier reports in monkeys and humans. The magnitude of ΔpB correlated highly with the baseline pB values in all divisions of the striatum. Based upon the principles of competitive binding, the slope of this empirical relationship, f_i, is equal to the fraction of [^{11}C]raclopride binding sites sensitive to endogenous dopamine; the magnitude of this fraction ranged from 0.29 in the caudate to 0.36 in the ventral striatum.

Keywords: [^{11}C]raclopride; Dopamine receptors; Pig; Amphetamine; Cortisol

1. Introduction

The availability of dopamine $D_{2/3}$ receptors for binding of [^{11}C]raclopride and other benzamide radioligands in living brain is influenced by competition from endogenous dopamine [33]. Thus, pharmacological challenge with D-amphetamine and other psychostimulants increases the extracellular dopamine concentration and consequently decreases the fraction of dopamine $D_{2/3}$ binding sites available for [^{11}C]raclopride binding in studies of mice [11], cats [22], monkeys [7,14,23], and humans (see [33]). Similarly, we have detected nicotine-induced reductions in [^{11}C]raclopride binding in the striatum of Danish Landrace pigs, which we attribute to pharmacological potentiation of dopamine release [10]. Corroborative PET studies in the monkey indicate that the amphetamine-induced reduction in [^{11}C]raclopride binding exceeds in magnitude that evoked by nicotine [38]. We have recently reported the effects of acute treatment with 3,4-

* Corresponding author. Tel.: +45 35283753; fax: +45 35282755/3535314.
E-mail addresses: nml@kvl.dk, nml@cnsr.dk (N.M. Lind).

0361-9230/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.brainresbull.2004.08.007

methylenedioxymethamphetamine on [^{11}C]raclopride binding in brain of living pig [42], but corresponding effects of D-amphetamine are unknown.

Female rodents are more sensitive than males to the psychomotor stimulant effects of amphetamines [3,43], a phenomenon, which has been linked to estrogen-mediated enhancement of the magnitude of dopamine release [8,49]. In PET studies of healthy humans, dopamine synthesis capacity is greater in striatum of women [32], whereas the apparent affinities for [^{11}C]raclopride suggest a higher basal occupancy of the $D_{2/3}$ receptors by dopamine in female brain [40]. However, it is unknown if gender differences influence the magnitude of amphetamine-evoked changes of [^{11}C]raclopride binding in living brain. Therefore, we recorded the cerebral uptake of [^{11}C]raclopride in baseline and post-amphetamine conditions in age-matched groups of six male and six female Göttingen minipigs and generated maps of the specific binding potential *(*pB*)* of [^{11}C]raclopride, which were then co-registered to a common stereotaxic coordinate system for minipig brain [47]. Statistical parametric mapping was used to test the hypothesis that [^{11}C]raclopride binding is more sensitive to D-amphetamine-evoked dopamine release in the female as compared to male pigs.

2. Materials and methods

2.1. PET methods

All procedures in this study were performed in accordance with the Danish Animal Experimentation Act (based on the Council of Europe Convention ETS 123) on a license granted by the Ministry of Justice. Six male and six female Göttingen minipigs (Dalmose, Denmark) aged 10 months and weighing 17–24 kg, were used in the PET study. Prior to entry into the PET study, the animals had been trained in a series of cognitive and behavioural tasks for a period of 6 months. During this time, each pig was treated with amphetamine (1 mg/kg, i.m.) four times. The final exposure to amphetamine had occurred 2 months prior to the PET study.

Pigs were acclimatized for a period of 1 week after transport to a facility in the vicinity of the PET Centre. On the day of PET scanning, pigs were sedated with 6–7 ml midazolam (5 mg/ml) for conveyance to the PET Centre. Deep anaesthesia was induced with i.v. midazolam (maximum 10 ml) and ketamine (maximum 8 ml). Following intubation, anaesthesia was maintained with isoflurane and N_2O (1:2). A Cook catheter (size 5.3) was placed in a femoral vein for administration of tracers and for blood collection. Animals were placed in dorsal recumbency, and the head was fixated using a custom-made head holder. After positioning of the head in the Siemens ECAT EXACT HR tomograph (6 mm × 6 mm × 6.5 mm full-width half-maximum intrinsic resolution), a brief attenuation scan was obtained using a rotating Ga^{2+} rod source. [^{11}C]raclopride of high-specific activity was synthesized using [^{11}C]methyl iodide and *o*-desmethylraclopride [12]. A dynamic emission recording sequence lasting 90 min was initiated after intravenous injection of 200–500 MBq [^{11}C]raclopride (377 ± 93 MBq). After completion of this recording, D-amphetamine sulfate (1 mg/kg [0.6 mg/kg free base], i.v., Sigma Chemicals) was administered as an infusion lasting 5 min. Ten minutes after completion of the infusion, a second dynamic [^{11}C]raclopride recording was made, identical to the first. Emission frames were reconstructed using filtered back-projection and an 8 mm Gaussian filter, resulting in frames with 128 × 128 pixels and 46 slices. After completion of the second recording, animals were returned to the home stable. One male animal had two separate determinations of baseline and post D-amphetamine [^{11}C]raclopride uptake, carried out 1 week apart.

Venous blood samples were drawn at baseline and at 30 and 60 min following amphetamine administration. Serum was isolated and stored at −80 °C, until analysis for content of cortisol, testosterone (males) and estradiol-17β (females) using direct chemiluminescence technology (ADVIA® Centaur™ Immunoassay system) at the Clinical Chemistry Department, Aarhus General Hospital.

The summed emission recording from scan 1 was manually aligned to a stereotaxic atlas of the brain from MR recordings made in 22 normal male Göttingen minipigs [47], using the program Register [37] with 9 degrees of freedom. Summed emission recordings from scan 1 and scan 2 were co-registered, and inspection of the fusion images was made to assess the head movement between scans. In 2 out of 13 cases, there was noticeable head movement, requiring an additional six-parameter transformation; the final transformation to stereotaxic space was calculated by concatenation of the two transformation matrices. Dynamic emission recordings were then resampled to the stereotaxic space and a conservative statistically-defined template for cerebellum ($0.75 < p < 1.0$) was applied to the dynamic recordings to extract a time–radioactivity curve for the cerebellum, a reference region assumed to contain no dopamine $D_{2/3}$ specific binding sites. The binding potential (pB) for [^{11}C]raclopride corresponds to the ratio B_{max}/K_d, reduced by competition from endogenous dopamine (see Appendix, Eq. (A.1)). Maps of the [^{11}C]raclopride pB were calculated voxel-wise using a reference tissue linearization [36], with exclusion of data points recorded in the first 10 min. For each animal, the post D-amphetamine pB map was subtracted from the baseline pB map to generate a difference map *(*ΔpB*)*. The mean magnitude of pB in the baseline and post-D-amphetamine conditions was calculated in seven volumes of interest (VOIs) defined in the stereotaxic atlas of minipig brain: left and right caudate, putamen, ventral striatum (encompassing the nucleus accumbens), and the whole thalamus. In these whole brain regions, the relationship between individual estimates of amphetamine-evoked ΔpB and the baseline pB was plotted, and the slope of this empirical relationship calculated by linear regression.