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Effects of unilateral 6-OHDA lesions on [^{3}H]-*N*-propylnorapomorphine binding in striatum ex vivo and vulnerability to amphetamine-evoked dopamine release in rat

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ABSTRACT

It has been argued that agonist ligands for dopamine $D_{2/3}$ receptors recognize a privileged subset of the receptors in living striatum, those which are functionally coupled to intracellular G-proteins. In support of this claim, the $D_{2/3}$ agonist [^{3}H]-*N*-propylnorapomorphine ([^{3}H]NPA) proved to be more vulnerable to competition from endogenous dopamine than was the antagonist ligand [^{11}C]raclopride, measured ex vivo in mouse striatum, and subsequently in multi-tracer PET studies of analogous design. Based on these results, we predicted that prolonged dopamine depletion would result in a preferential increase in agonist binding, and a lesser competition from residual dopamine to the agonist binding. To test this hypothesis we used autoradiography to measure [^{3}H]NPA and [^{3}H]raclopride binding sites in hemi-parkinsonian rats with unilateral 6-OHDA lesions, with and without amphetamine challenge. Unilateral lesions were associated with a more distinct increase in [^{3}H]NPA binding ex vivo than was seen for [^{3}H]raclopride binding in vitro. Furthermore, this preferential asymmetry in [^{3}H]NPA binding was more pronounced in amphetamine treated rats. We consequently predict that agonist ligands should likewise be fitter than antagonists for detecting responses to denervation in positron emission tomography studies of idiopathic Parkinson's disease. Agonist binding increases in vivo are likely to reflect the composite of a sensitization-like phenomenon, and relatively less competition from endogenous dopamine, as seen in the lesioned side of 6-OHDA induced hemi-parkinsonism.

1. Introduction

In molecular imaging studies employing a competition paradigm, in vivo changes in the availability of binding sites for benzamide antagonists of dopamine $D_{2/3}$ receptors are interpreted to reveal altered competition from endogenous dopamine (Laruelle, 2000). However, dopamine receptors can exist in distinct affinity states with respect to dopamine and exogenous agonist ligands, with perhaps 50% of the receptors, designated D_2^{High}, naturally occurring in a state with high affinity for agonists, and coupled with intracellular G-proteins. As such, the fractional abundance of D_2^{High} sites would limit the possible extent of occupancy by endogenous dopamine, and therefore should also represent the upper limit of the vulnerability of the in vivo binding of benzamide dopamine antagonists to competition from endogenous dopamine or exogenous agonists. In support of this notion, the dopamine $D_{2/3}$ agonist [^{3}H]-*N*-propylnorapomorphine [^{3}H]NPA proven to be considerably more sensitive than was the antagonist [^{11}C]raclopride to pharmacologically evoked changes in dopamine tonus in striatum of living mice (Cumming et al., 2002) and likewise in positron emission tomography (PET) agonist studies of non-human primate (Narendran et al., 2005). However, the potential utility of agonist ligands for PET investigations of the pathophysiology of Parkinson's disease has scarcely been investigated.

Parkinson's disease can be modeled in rats, which have undergone substantial depletions of the dopamine innervation in striatum following unilateral infusion of the toxin 6-OHDA to the medial forebrain bundle. In general, these dopamine depletions eventually result in increased abundance of binding sites for dopamine D_2-like receptor antagonists, as in untreated cases of

Abbreviations: NSB, non specific binding; ROI, region of interest; SBR, specific binding ratio.

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idiopathic Parkinson's disease. Thus, molecular imaging has revealed a 40% increase in [^{11}C]raclopride binding in striatum of untreated patients with Parkinson's disease (Dentresangle et al., 1999). The response of agonist binding sites to dopamine depletion is less well-established. The specific binding of [^{3}H]NPA was 50–100% increased in rat striatum ipsilateral to extensive 6-OHDA lesions (Van der Werf et al., 1984). However, in untreated patients with early Parkinson's disease, the binding in striatum (more exactly the putamen) of the D_3-prefering agonist [^{11}C]PHNO was elevated by 25%, as was likewise the binding of [^{11}C]raclopride (Boileau et al., 2009). Furthermore, the vulnerability of [^{11}C]PHNO binding in intact striatum does not differ from that of [^{3}H]raclopride in an amphetamine challenge study ex vivo (McCormick et al., 2008), which might likewise predict a comparable responsiveness of agonist sites to 6-OHDA lesions as well. Thus, it has not been clearly established that agonist binding sites are up-regulated to a greater extent than antagonist sites in a condition of dopamine depletion. Furthermore, the studies have hitherto considered whole striatum, or large divisions such as the putamen. Therefore, in order to compare the responsiveness of agonist and antagonist binding sites to prolonged dopamine depletion, we used quantitative autoradiography to measure ex vivo the availability of binding sites for [^{3}H]NPA in five divisions of the extended striatum of rats with unilateral 6-OHDA lesions. These results were directly contrasted with the density of binding sites measured with [^{3}H]raclopride in vitro from the same animals. We also tested the hypothesis that [^{3}H]NPA binding ex vivo should be asymmetrically responsive to amphetamine challenge, in analogy to a pharmacological activation study in early Parkinson's disease, which is usually characterized by asymmetric nigrostriatal degeneration. The [^{3}H]raclopride autoradiography in vitro was conducted in order to quantify the up-regulation of antagonist binding sites in divisions of the denervated striatum.

2. Materials and methods

2.1. Materials

Chemicals was obtained from Sigma–Aldrich except in the following cases: zoletil (Virbac), xylazine (Intervet), butorphanol tartrate (Scan Vet), d-amphetamine (Lipomed), [^{3}H]NPA (Vitrax), [^{3}H]raclopride (PerkinElmer) and [^{123}I]PE2I (MAP Medical Technologies).

2.2. Animals

Sixteen male Sprague–Dawley rats (Charles River, Germany) weighing between 250 and 300 g were used in this study. The animals were held under standard laboratory conditions with a 12-h light/12-h dark cycle and ad libitum access to food and water. After arrival, the animals were allowed to acclimatize for at least five days before use. All efforts were made to minimize animal suffering, to reduce the number of animals used. All animal experiments were carried out in accordance with the European Communities Council Resolves of 24 November 1986 (86-609/ECC) and approved by the Danish State Research Inspectorate (J. No. 2002/561-527).

2.3. 6-OHDA lesion

The rats were anesthetized with 1 mL/kg i.m. of a mixture containing zoletil (6.25 mg/mL), xylazine (5 mg/mL) and butorphanol tartrate (0.25 mg/mL). Animals then received desmethylimipramine (DMI) (25 mg/kg, i.p.) to protect noradrenergic neurons, and were placed in a stereotaxic apparatus (David Kopf Instruments) with the incisor bar set 3.3 mm below the level of the ear bars. A solution of 6-OHDA hydrobromide (8 μg dissolved in 4 μL saline containing 0.05% ascorbic acid) was drawn into a 10 μL syringe, and infused into the medial forebrain bundle at coordinates (AP: −4.4 mm, ML: 2 mm, DV: 8 mm) according to the coordinates given by Paxinos and Watson. The infusion was delivered at 1 μL/min driven by an infusion pump, followed by a 5 min pause prior to slow withdrawal of the syringe needle. The burr hole was closed with bone wax and the wound was sutured. After recovery from anesthesia, rats were returned to the home cage for recovery for 14 days, so as to allow development of the lesions, and the attainment of stable changes in post-synaptic dopamine $D_{2/3}$ receptors in response to prolonged denervation.

2.4. Ex vivo [^{3}H]NPA autoradiography and response to amphetamine

Rats were randomly assigned to saline or amphetamine groups. During acute immobilization, saline ($n = 9$) or d-amphetamine ($n = 7$) (3 mg/kg, in a total of 500 μL saline) was administered i.p. 5 min prior to tracer injection. The [^{3}H]NPA (specific activity >50 Ci/mmol) was administered through the tail vein as a single bolus in 300 μL saline containing 0.1% ascorbic acid. Rats were returned to their cage, and killed by decapitation 45 min later, doing a state of pseudoequilibrium of the specific [^{3}H]NPA binding (Cumming et al., 2002). Brains were quickly removed, rinsed in ice-cold water, and placed on ice until frozen by immersion in isopentane at −40 °C. After storage at −80 °C, brains were cut with a cryostat (Microme HM 500 OM), and 20 μm sections, collected from the anterior striatum (+1.56 to +1.36 AP) and posterior striatum (−1.08 to −1.28 AP), were mounted on glass slides. Those slides for autoradiography ex vivo were dehydrated and fixed overnight by exposure to para-formaldehyde vapor. The following day, they were placed along with tritium standard strips (Amersham) on phosphor imaging plates (BAS-TR2040, Fujifilm), which were exposed for three weeks. We calculated the specific binding ratio (SBR) in each region of interests (ROI), as defined in Fig. 1. Non-specific binding (NSB) was determined by the binding in cortex on the same section. SBR = (total activity in ROI − total activity in NSB ROI)/total activity in NSB ROI. The binding in the lesioned side was reported (Fig. 2) as a percentage of the SBR in the lesion side to the corresponding SBR in the intact side.

2.5. In vitro [^{3}H]raclopride autoradiography

Sections collected from the ex vivo experiment were washed 2× 15 min in assay buffer (Tris–HCl 50 mM, NaCl 150 mM, KCl 5 mM, $CaCl_2$ 1 mM, $MgCl_2$ 1 mM, 0.1% ascorbic acid, pH 7.4, 21 °C) to remove [^{3}H]NPA and endogenous dopamine. Sections was then incubated for 60 min at room temperate in assay buffer modified by the addition of [^{3}H]raclopride to a final concentration of 2 nM. For every second section, butaclamol (10 μM) was added to determine the non-specific binding. After 60 min the incubation was terminated by washing 3× 5 min in ice cold assay buffer. The sections was dehydrated, fixed in para-formaldehyde vapor and exposed to an imaging plate (BAS-TR2040, Fujifilm) for 14 days, and the binding in lesioned vs. intact sides was calculated as above.

2.6. Dopamine transporter [^{123}I]PE2I autoradiography

In order to verify the 6-OHDA lesions, selected brain sections from anterior and posterior levels were incubated at room temperature in Tris buffer (pH 7.4) containing 200 mM NaCl for 10 min prior to addition of the dopamine transporter ligand, [^{123}I]PE2I (8.8 TBq/μmol) to a final concentration of 2 nM. After 60 min incubation at room temperature, the slides were washed in ice-cold buffer (3× 5 min), rapidly dried, and then exposed to an imaging plate (BAS-MS 2040, Fujifilm) for 14 h, and the binding in lesioned vs. intact sides was calculated as above.

2.7. Statistics

ImageJ v1.37 was used for image analysis. A paired two-tailed *t*-test was used to compare lesion vs. healthy side on the same section, an unpaired two-tailed *t*-test was used to compare across sections and an unpaired one tailed *t*-test was used to calculate the significance of a % increase. A two-tailed ANOVA was used to calculate the significance of the gradient in striatum. Significant differences was marked as $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$. A few significant outliers were removed after a Grubb's test with confidence interval of 99%. All deviations are shown as standard errors of the mean (SEM).

3. Results

Representative autoradiograms for [^{125}I]PE2I, [^{3}H]NPA and [^{3}H]raclopride are shown in Fig. 1. [^{125}I]PE2I specific binding was 90% abolished in striatum on the ipsilateral side of the lesion, whereas the extent of the lesion was less consistent in the nucleus accumbens (30 ± 81%). The binding (SBR) of [^{3}H]NPA was increased by approximately 50% in the lesioned anterior striatum (anterior dorsal medial and anterior ventral lateral) of the saline treated rats and by 100% in the same regions of the amphetamine-treated rats (Fig. 2). Lesser, but not significant, increases in binding (SBR) were seen in lesioned nucleus accumbens and posterior striatum (posterior dorsal medial and posterior ventral lateral). Increases in the binding (SBR) of [^{3}H]raclopride on the lesioned side were in the range of 0–20% in both saline and amphetamine groups, reaching significance only in the posterior ventral lateral region of striatum.

The overall percentage increases in binding were greater with [^{3}H]NPA than [^{3}H]raclopride, a difference reaching significance in