Pharmacology, Biochemistry and Behavior 153 (2017) 76–87

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

GABAergic control of neostriatal dopamine D_2 receptor binding and behaviors in the rat

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ARTICLE INFO

Article history:
Received 11 November 2016
Received in revised form 15 December 2016
Accepted 19 December 2016
Available online 21 December 2016

Keywords:
D_2 receptor
[^{123}I]IBZM
$GABA_A$ receptor
Muscimol
Bicuculline
Time-behavior curves
Small animal SPECT

ABSTRACT

Purpose: The present study assessed the influence of the $GABA_A$ receptor agonist muscimol and the $GABA_A$ receptor antagonist bicuculline on neostriatal dopamine D_2 receptor binding in relation to motor and exploratory behaviors in the rat.
Methods: D_2 receptor binding was measured in baseline and after challenge with either 1 mg/kg muscimol or 1 mg/kg bicuculline. In additional rats, D_2 receptor binding was measured after injection of saline. After treatment with muscimol, bicuculline and saline, motor and exploratory behaviors were assessed for 30 min in an open field prior to administration of [^{123}I]S-3-iodo-*N*-(1-ethyl-2-pyrrolidinyl)methyl-2-hydroxy-6-methoxybenzamide ([^{123}I]IBZM). For baseline and challenges, striatal equilibrium ratios (V_3'') were computed as estimation of the binding potential.
Results: Muscimol but not bicuculline reduced D_2 receptor binding relative to baseline and to saline. Travelled distance, duration of rearing and frequency of rearing and of head-shoulder motility were lower after muscimol compared to saline. In contrast, duration of rearing and grooming and frequency of rearing, head-shoulder motility and grooming were elevated after bicuculline relative to saline. Moreover, bicuculline decreased duration of sitting and head-shoulder motility.
Conclusions: The muscimol-induced decrease of motor/exploratory behaviors can be related to an elevation of striatal dopamine levels. In contrast, bicuculline is likely to elicit a decline of synaptic dopamine, which, however, is compensated by the time of D_2 receptor imaging studies. The results indicate direct GABAergic control over D_2 receptor binding in the neostriatum in relation to behavioral action, and, thus, complement earlier pharmacological studies.

1. Introduction

The neostriatum (STR) receives glutamate(GLU)ergic afferents from neocortex (motor, somatosensory and frontal/prefrontal cortex) and thalamus (THAL) as well as dopamin(DA)ergic ones from ventral tegmental area (VTA) and the pars compacta of the substantia nigra (SNc; for review see Strüder and Weicker, 2001). In turn, it sends DAergic efferents back to the SNc (for review see Gerfen, 1984) as well as to THAL and motor cortex (for review see Strüder and Weicker, 2001). The nigrostriatal DAergic system is under inhibitory γ-amino butyric acid (GABA)ergic control (for review see Gale and Casu, 1981; Sivilotti and Nistri, 1991). From the STR, GABAergic neurons project either directly or via the external part of the globus pallidus (GPe) and the subthalamic nucleus (STN) to the internal part of the globus pallidus (GPi) and to the pars reticulata of the SN (SNr), which send GABAergic efferents to THAL, inferior and superior colliculus, pedunculopontine nucleus and periaqueductal grey (Strüder and Weicker, 2001; Coimbra and Brandao, 1993). Further inhibitory action is exerted by neostriatal GABAergic microcircuits, which are formed by fast-spiking interneurons and collaterals of descending projection neurons (for review see Tepper et al., 2004).

Muscimol (MUS; 5-aminomethyl-isoxazol-3-ol) and bicuculline (BIC; (6R)-6-[(5S)-6-methyl-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-g]isoquinolin-5-yl]furo[3,4-e][1,3]benzodioxol-8(6H)-one) act as an highly selective $GABA_AR$ agonist (inhibition constant [K_i] = 2.7 nM, Negro et al., 1995) and $GABA_AR$ antagonist (K_i = 3.04 nM, Ito et al., 1988), respectively. Intracerebral effects of MUS and BIC on extracellular DA levels are region-specific: MUS infusions into the prefrontal cortex (PFC; 0.1 and 1 mM) decreased synaptic DA in the STR (Matsumoto et al., 2003). Similarly, application of MUS into the VTA (10–40 μM, Westerink et al., 1996; 20 μM, Westerink et al., 1998) reduced DA concentrations in the nucleus accumbens (NAC; Westerink et al., 1996) and in the PFC (Westerink et al., 1998). In contrast, intranigral infusion

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http://dx.doi.org/10.1016/j.pbb.2016.12.012
0091-3057/

of MUS (10 μM) led to an increase of neostriatal DA concentrations (Santiago and Westerink, 1992). Likewise, an elevation of synaptic DA was observed in the SN upon infusion of MUS (100 μM) into the GP (Cobb and Abercrombie, 2002, 2003). Moreover, MUS administration into VTA (10 and 100 μM, Klitenick et al., 1992) and NAC (50 μM, Yoshida et al., 1997; 2.5 nM, Aono et al., 2008) augmented DA levels in these regions. No effect on prefrontal DA levels was obtained after infusion of 50 and 500 μM MUS into the PFC (Santiago et al., 1993).

BIC infusions into the PFC (50 and 100 μM, Karreman and Moghaddam, 1996; 30 and 100 μM, Matsumoto et al., 2003) increased DA levels in the STR. Likewise, application of BIC into the SN (50 μM, Santiago and Westerink, 1992; Westerink et al., 1992) and VTA (200 μM, Ikemoto et al., 1997) elevated DA concentrations in both SN and STR (Santiago and Westerink, 1992; Westerink et al., 1992) as well as in NAC (Ikemoto et al., 1997). Similarly, infusions into the STR (100 μM, Smolders et al., 1995) and the NAC (25, 50 or 100 μM, Yan et al., 1999; 50 pmol, Aono et al., 2008) augmented DA levels in STR and NAC, respectively.

Upon infusion into VTA or SN, MUS (88 μM, Scheel-Krüger et al., 1977; 54 or 136 μM, Arnt and Scheel-Krüger, 1978; 0.175–3.5 mM, Oakley et al., 1991; 10–1000 μM, Klitenick et al., 1992; 525 μM, Trevitt et al., 2002) was found to elevate locomotion and exploratory activities in the rat such as rearing and sniffing. Similarly, BIC increased locomotion, when applied into zona incerta (136 nM and 272 nM, Périer et al., 2002), THAL (30 and 100 μM, Bubser et al., 1997) and ventral pallidum or lateral preoptic area (0.136 or 27 mM, Reynolds et al., 2006; 730 μM, Zahm et al., 2014). However, a reduction of motor activity was found after infusion of BIC into the SN (65 μM and 97 μM, Trevitt et al., 2002). Furthermore, injections of BIC into THAL (Bubser et al., 1997) as well as STN or zona incerta (Périer et al., 2002) elevated rearing behavior, but diminished sniffing and grooming.

Disturbances of $GABA_AR$ function are known to be associated with a variety of psychiatric conditions including schizophrenia, anxiety disorders and autism spectrum disorders (for reviews see Nikolaus et al., 2010, 2014a; Cellot and Cherubini, 2014). So far, effects of MUS and BIC on striatal DA have not been assessed using small animal imaging methods. We have previously shown that D_2R imaging can be employed in order to estimate alterations of synaptic DA upon challenge with compounds such as methylphenidate and L-DOPA, which modify DA availability in the synaptic cleft (Nikolaus et al., 2005, 2011, 2016a, 2016b). In the present study, we set out to assess the effect of MUS and BIC on behavior and D_2R binding in the rat STR. To permit comparisons between treatment groups, behavior and D_2R binding were also assessed in saline(SAL)-treated rats. In analogy to our previous studies of D_2R binding after methylphenidate and L-DOPA (Nikolaus et al., 2005, 2011, 2016a, 2016b), rats underwent systemic application of MUS or BIC. D_2R binding data in baseline (BAS) and after challenge with either 1 mg/kg MUS or 1 mg/kg BIC were used to estimate alterations of synaptic DA. Moreover, temporal dynamics of behavior after MUS, BIC and SAL were assessed by fitting suitable models to the acquired data and by statistically comparing time-behavior (t-b) curves. The benefit of this approach (Nikolaus et al., 2014b, 2016a) is the gaining of information on the temporal dynamics of behavior, which is not achieved by the standard dose-response curves relating changes in behavior merely to dose but not to time.

2. Materials and methods

2.1. Animals

We used a total of 48 adult male Wistar rats (ZETT, Heinrich-Heine University, Düsseldorf, Germany), weighing 429 ± 42 g (mean ± standard deviation [SD]). Of these, 32 animals underwent D_2R imaging in BAS and both D_2R imaging and behavioral testing after treatment with MUS or BIC ($n = 16$, respectively). One animal merely underwent behavioral measurements after MUS challenge without imaging, since it dropped out after administration of anaesthesia. Sixteen further rats were subjected to behavioral measurements and D_2R imaging after treatment with vehicle (0.9% SAL). Two of these died after anaesthesia, and, therefore, only underwent behavioral testing. Imaging and behavioral data obtained after SAL were previously published (Nikolaus et al., 2016a, 2016b). Rats were maintained in standard macrolon cages (590 × 380 × 200 mm; 3 animals per cage) in a climate cabinet (Scantainer, Scanbur BK, Karslunde, Denmark; temperature, 20 °C; air humidity, 70%) with an artificial light-dark cycle (lights on at 6:00 a.m., lights off at 6:00 p.m.) and food and water freely available. The study was approved by the regional authority and carried out in accordance with the "Principles of laboratory animal care" (NIH publication no. 86–23, revised 1985) and the German Law on the Protection of Animals.

2.2. SPECT camera

The small animal tomograph ("TierSPECT") was described in detail elsewhere (Schramm et al., 2000). For ^{123}I, tomographic resolution and sensitivity amounted to 3.4 mm and 16 cps/MBq, respectively. A low-energy ultra-high-resolution parallel-hole collimator (LEUHR, $37 \times 1 \times 0.2$ mm^3) was mounted in front of the detector head. Data were acquired in a 128×128 matrix with a pixel width and a slice thickness of ≈0.664 mm, respectively. Acquisition was conducted for 60 min in a step-and-shoot mode over a circular orbit in angular steps of 6° (60 projections, 60 s/projection). Data were reconstructed with an iterative ordered-subset-expectation-maximization algorithm (3 iterations, 4 subsets/iteration). No post-filtering procedure was applied. An attenuation correction of 0.11 cm^{-1} was implemented assuming a uniformly attenuating medium.

2.3. D_2 receptor imaging studies

D_2R binding was assessed in BAS and after intraperitoneal (i.p.) injections of MUS (Sigma-Aldrich, Taufkirchen, Germany; molecular weight: 114.1 g/mol, dose: 1 mg/kg, concentration: 1 mg/ml) or BIC (1(*S*),9(*R*)-(−)-bicuculline methchloride; Sigma-Aldrich, Taufkirchen, Germany; molecular weight: 367.35 g/mol, dose: 1 mg/kg, concentration: 1 mg/ml). Additional rats underwent measurement of D_2R binding after i.p. injection of vehicle (0.9% SAL; B. Braun Melsungen AG, Melsungen, Germany; dose: 1 ml/kg). Measurements in BAS and after challenge (either MUS or BIC) were performed in randomized order. The animals in the control group merely underwent one measurement of D_2R binding after challenge with SAL.

The applied doses of MUS and BIC had been previously shown to be behaviorally active after systemic application (e.g., Corbett et al., 1991; Zhang and Cranney, 2008). In addition, the 1 mg/kg dose of BIC had been proven subconvulsant (Girardi and Rodríguez de Lores Arnaiz, 1987). In previous studies, maximum motor and exploratory activities were observed at 10 to 30 min after intracerebral application of MUS (Oakley et al., 1991), whereas, after BIC, maximum motor and exploratory activities occurred from 2 min post-injection onward and then declined over the following 20 min (Zahm et al., 2014). As a consequence, behavioral measurements were started immediately post-challenge and performed for a total of 30 min.

Thirty min after MUS, BIC or SAL, animals received i.p. injections of 0.9 ml/kg ketaminehydrochloride (Ketavet®, Pharmacia GmbH, Erlangen, Germany; concentration: 100 mg/ml) and 0.4 ml/kg xylazinehydrochloride (Rompun® Bayer, Leverkusen, Germany; concentration: 0.02 mg/ml). Then 25.4 ± 3.6 MBq [^{123}I]S-3-iodo-*N*-(1-ethyl-2-pyrrolidinyl)methyl-2-hydroxy-6-methoxybenzamide ([^{123}I]IBZM; GE Healthcare, München, Germany; concentration: 3.4×10^{-9} g/ml, specific activity: >74 TBq/mmol at reference time) were injected into the lateral tail vein using a winged infusion needle set. The tube was rinsed with 1 ml 0.9% SAL, amounting to a total injection volume of 1.3 ml.