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Cocaine self-administration differentially affects allosteric A2A-D2 receptor-receptor interactions in the striatum. Relevance for cocaine use disorder

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ABSTRACT

In the current study behavioral and biochemical experiments were performed to study changes in the allosteric A2AR-D2R interactions in the ventral and dorsal striatum after cocaine self-administration versus corresponding yoked saline control. By using ex vivo [^{3}H]-raclopride/quinpirole competition experiments, the effects of the A2AR agonist CGS 21680 (100 nM) on the Ki_H and Ki_L values of the D2-like receptor (D2-likeR) were determined. One major result was a significant reduction in the D2-likeR agonist high affinity state observed with CGS 21680 after cocaine self-administration in the ventral striatum compared with the yoked saline group. The results therefore support the hypothesis that A2AR agonists can at least in part counteract the motivational actions of cocaine. This action is mediated via the D2-likeR by targeting the A2AR protomer of A2AR-D2-likeR heteroreceptor complexes in the ventral striatum, which leads to the reduction of D2-likeR protomer recognition through the allosteric receptor-receptor interaction. In contrast, in the dorsal striatum the CGS 21680-induced antagonistic modulation in the D2-likeR agonist high affinity state was abolished after cocaine self-administration versus the yoked saline group probably due to a local dysfunction/disruption of the A2AR-D2-likeR heteroreceptor complexes. Such a change in the dorsal striatum in cocaine self-administration can contribute to the development of either locomotor sensitization, habit-forming learning and/or the compulsive drug seeking by enhanced D2-likeR protomer signaling. Potential differences in the composition and stoichiometry of the A2AR-D2R heteroreceptor complexes, including differential recruitment of sigma 1 receptor, in the ventral and dorsal striatum may explain the differential regional changes observed in the A2A-D2-likeR interactions after cocaine self-administration.

1. Introduction

Adenosine A2A receptor (A2AR)-dopamine (DA) D2 receptor (D2R) heteroreceptor complexes with antagonistic allosteric receptor-receptor interactions are well-known to exist in the ventral and dorsal striatum (Fuxe et al., 1998; Trifilieff et al., 2011; Borroto-Escuela et al., 2013; Fuxe et al., 2014). Such antagonistic allosteric A2AR-D2R interactions have been demonstrated at the neurochemical levels (Wydra et al., 2013; Frankowska et al., 2013; Pettit and Justice, 1989; Lecca et al., 2007) and in the behavioral models, including cocaine reward and cocaine seeking in animals (Filip et al., 2006, 2012; Wydra et al., 2015a, 2013; O'Neill et al., 2014, 2012). In the ventral striatum of rats self-administering cocaine, no changes were found in the density and Kd values of A2AR and D2-like receptor (D2-likeR) in biochemical radioligand binding experiments with the D2-likeR antagonist [^{3}H]-raclopride and the A2AR antagonist [^{3}H]-ZM 241385 (Frankowska et al., 2013). Interestingly, the findings from the dorsal striatum of rats self-administering cocaine mirror those found in the ventral striatum, with the exception of an increased affinity found in the A2AR antagonist binding sites using saturation biochemical binding (Frankowska et al., 2013). However the major change induced by cocaine self-administration in the dorsal striatum was an increased potency of DA to increase the D2R-Gi/o coupling as studied in the GTPγS accumulation assay, not demonstrated in the ventral striatum (Frankowska et al., 2013).

Our current follow-up work focused on the allosteric A2AR-D2-likeR interactions using the A2AR agonist CGS 21680 to modulate the high and low affinity dissociation constants (Ki_H and Ki_L) of the D2-likeR agonist binding sites obtained from competition curves in the ventral and dorsal

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striatum determined ex vivo after cocaine self-administration sessions. For the first time it is reported that cocaine self-administration in rats induces alterations in the antagonistic allosteric A2AR-D2-likeR interactions that developed differentially in the ventral versus the dorsal striatum.

2. Materials and methods

2.1. Animals

The experiments were carried out in accordance with the European *Directive* 2010/63/*EU* and were approved by the Bioethical Committee at the Institute of Pharmacology, Polish Academy of Sciences, Krakow. Male Wistar rats (derived from the licensed animal breeder Charles River, Sulzfeld, Germany), weighing between 260 and 310 g at the beginning of the experiment were used. The animals were housed individually in standard plastic rodent cages (25 cm × 30 cm × 30 cm) in a colony room maintained at 21 ± 1 °C and 40–50% humidity under a 12-hour light-dark cycle (lights on at 6:00 am). Rodent food and water were available ad libitum except for the period of the initial training sessions when rats were maintained on limited water (see below). All protocols were conducted during the light phase of the light-dark cycle between 9:00 and 13:00 h. All animals used in the study were experimentally naive.

2.2. Drug

Cocaine hydrochloride (Sigma-Aldrich; St. Louis, USA) was dissolved in sterile 0.9% NaCl and was given intravenously (i.v.; 0.1 ml/infusion).

2.3. Behavioral experiments

Behavioral experiments included acquisition and maintenance of intravenous cocaine self-administration with the corresponding yoked saline control.

2.3.1. Cocaine self-administration

After initial training to press levers during which the amount of water that each animal received was restricted to that given during daily 2 h training sessions, animals with free access to water were implanted with a silastic® catheter in the external jugular vein, as described previously (Wydra et al., 2013). Catheters were flushed daily with 0.1 ml of a heparinized saline solution (70 U/ml, Biochemie, Austria) and 0.1 ml of a cephazolin solution (10 mg/ml Biochemie GmbH, Austria). Catheter patency was tested periodically or whenever an animal displayed behavior beyond baseline parameters methohexital was used (10 mg/kg, i.v.), which induced the loss of consciousness within 5 s. No problems with catheter patency were reported in the tested rats. Rats were allowed 10 days to recover from surgery before the start of the experiments. Later on, all animals began lever pressing for cocaine reinforcement during 2 h daily sessions performed 6 days per week. Each completion of a fixed ratio (FR) 5 schedule on the "active" lever resulted in a 5 s injection of cocaine (0.5 mg/kg per infusion) together with a presentation of conditioned stimulus (light + tone). Following each injection, there was a 20 s time-out period during which responding was recorded, but had no programmed consequences. Response on the "inactive" lever never resulted in cocaine delivery. Acquisition of the conditioned operant response lasted a minimum of 13–16 days until subjects met the following criteria: minimum requirement of 25 reinforcements and active lever presses as an average over 6 consecutive days and a standard value across those 6 days that varied by no more than 10% (Frankowska et al., 2013).

2.3.2. Yoked procedure

A "yoked" procedure (in which rats were tested simultaneously in groups of two, with one rat actively self-administering cocaine and the second receiving vehicle) was used.

2.4. Biochemical experiments

2.4.1. Membrane preparation

After the 13–16 days of cocaine self-administration and yoked saline delivery the rats were decapitated. The tissue was harvested immediately after the last 2 h cocaine self-administration session. The dorsal and ventral striatum were dissected out and immediately frozen on dry ice and stored at −80 °C. Frozen rat striata was homogenized in ice-cold preparation buffer using a sonicator (Soniprep 150). The buffer contained 50 mM Tris-HCl, pH 7.4, 7 mM $MgCl_2$, 1 mM EDTA and a cocktail of protease inhibitors (Roche Diagnostics, Mannheim, Germany). The membranes were precipitated by centrifugation at 4 °C for 40 min at 40,000×*g* (Thermo scientific, Sorvall Lynx 6000, Stockholm, Sweden) and washed through rehomogenization in the same buffer once more. The protein concentration was determined for the membrane homogenates by means of BCA Protein Assay (Pierce, Sweden) using as a standard bovine serum albumin (BSA). Pelleted membranes were resuspended to a concentration of 0.4 mg/ml, immediately used or stored at −80 °C until required.

2.4.2. [^{3}H]-Raclopride binding experiments

Competition experiments of quinpirole (0.3 nM–3 mM) versus the D2-likeR antagonist [^{3}H]-raclopride (2 nM; specific activity 78.1 Ci/mmol, PerkinElmer Life Sciences, Stockholm, Sweden) were carried out by membrane (20 μg per well, that were treated with adenosine deaminase for 30 min on ice) incubation at 30 °C for 90 min, in the presence or absence of 100 nM of the A2AR agonist CGS 21680. Nonspecific binding was defined by radioligand binding in the presence of 10 μM (+)-butaclamol (Sigma-Aldrich, Stockholm, Sweden). The incubation was terminated by rapid filtration through Hydrophilic (LPB) Durapore ®Membrane (Millipore, Stockholm, Sweden) using a MultiScreen™ Vacuum Manifold 96-well (Millipore Corp, Bedford, MA), followed by five washes (200 μl per wash) with ice-cold washing buffer (50 mM Tris-HCl pH 7.4). The filters were dried, 4 ml of scintillation cocktail was added and the bound ligand was determined after 12 h by liquid scintillation spectrometry.

2.5. Statistical analysis

All the data are shown as means ± SEM. In behavioral experiments, the number of responses on the active and inactive lever was analyzed using a one-way analysis of variance (ANOVA) for repeated measurements, the latter analysis followed by post hoc Tukey's test. The number of rats (*n*) in each experimental condition is indicated in figure legends. Data from the competition experiments were analyzed by nonlinear regression analysis using a commercial program GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA). pKi_H and pKi_L values from several replications in each rat are expressed as means ± SEM. The pKi_H (negative logarithm of the Ki) values are given in Table 1 and pKi_L values in Supplementary Table 1. The effects of CGS 21680 on these values and on the percent proportion of D2R agonist binding sites in the high affinity state (RH) were evaluated with paired Student's *t*-test and nonparametric Mann-Whitney *U* test, respectively. The percent change induced by CGS 21680 in the Ki_H and Ki_L values and in the percent proportion of D2-likeR in rats self-administering cocaine versus the yoked saline controls was compared using Mann-Whitney *U* test. The *P* value 0.05 and lower was considered significant.