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Alterations in ventral and dorsal striatal allosteric A2AR-D2R receptor-receptor interactions after amphetamine challenge: Relevance for schizophrenia

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ABSTRACT

Striatal dopamine D2R homodimerization is increased in the dorsal striatum after acute amphetamine challenge and in the amphetamine-induced sensitized state, a well-known animal model of schizophrenia. Therefore, it was tested if the increase in D2R homoreceptor complexes found after acute amphetamine challenge in the saline or the amphetamine sensitized state leads to changes in the antagonistic adenosine A2AR-D2R interactions in the striatum. [^{3}H]-raclopride binding was performed in membrane preparations from the ventral and dorsal striatum involving competition with the D2R like agonist quinpirole. In the ventral striatum CGS 21680 produced a significant increase of the K_{iH} values ($p < 0.05$) in the amphetamine sensitized group when expressed in percent versus the corresponding values in saline sensitized rats after amphetamine challenge. However, in the dorsal striatum a significant change did not develop in the K_{iH} values when expressed in percent of the corresponding values in saline sensitized rats after amphetamine challenge. In fact, the non-significant change was in the opposite direction towards a reduction of the K_{iH} values. Taken together, a reduced affinity of the high affinity D2 agonist binding site (K_{iH} value) developed in the ventral but not in the dorsal striatum as a result of increased antagonistic allosteric A2AR-D2R interactions in the amphetamine-induced sensitized state versus the saline sensitized state after an acute amphetamine challenge. The selective reappearance of antagonistic A2AR-D2R receptor-receptor interactions in the ventral striatum after amphetamine challenge in the amphetamine sensitized rat may give one possible mechanism for the atypical antipsychotic-like actions of A2AR receptor agonists.

1. Introduction

Blockade of dopamine D2 receptors (D2R) in the brain is the major therapeutic action of current antipsychotic drugs [20,22]. Fang Liu and colleagues recently reported that striatal D2R homodimerization is increased in the dorsal striatum after acute amphetamine challenge and in the amphetamine-induced sensitized state, a well-known animal model of schizophrenia [27]. This increase was also found in postmortem striatal tissue of schizophrenic patients. In ^{3}H-domperidone/DA competition experiments performed in this study the proportion of striatal D2Rs in the high affinity state was substantially increased. This is in line with previous findings of elevated $D2^{high}$ receptors in models of schizophrenia [20,21]. Furthermore, antagonistic adenosine A2AR-D2R receptor-receptor interactions were demonstrated in heteroreceptor complexes of the ventral and dorsal striatum and A2AR agonists possess atypical antipsychotic properties [2,10,12,26]. It was proposed that the D2R and A2AR homoreceptor complexes exist in balance with A2AR-D2R heteroreceptor complexes in ventral and dorsal striato-pallidal GABA neurons [3,7,12].

It is therefore of interest to study if the increase in D2R homoreceptor complexes found in the dorsal striatum after acute amphetamine challenge in the saline or the amphetamine sensitized state leads to changes in the antagonistic A2AR-D2R receptor-receptor interactions which are known to produce a reduction in the affinity of the high affinity D2R agonist binding site [8]. The relevance of such studies is further increased by the demonstration that cocaine self-administration increases the antagonistic A2AR-D2R interactions in the ventral striatum but switches them into facilitatory interactions in the dorsal striatum [17]. Experiments on A2AR-D2R receptor-receptor interactions were performed in the current study using the same amphetamine sensitization model as employed before [27] followed by an acute amphetamine challenge to document the sensitization development. A comparison was made between the ventral and dorsal striatum since

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the DA hypothesis of schizophrenia postulates a major involvement of a subcortical limbic hyperdopaminergia mediated via D2Rs [9]. This action modulates the brain circuit from the nucleus accumbens to the prefrontal cortex over the ventral pallidum and the mediodorsal thalamic nucleus and brings down the glutamate drive to the prefrontal cortex from the mediodorsal thalamic nucleus.

2. Experimental procedures

2.1. Animals

All studies involving animals were performed in accordance with guidelines from the Canadian Council on Animal Care (CCAC) and EU guidelines for accommodation and care of Laboratory Animals (appendix A). Male Sprague-Dawley rats, 8 weeks old, weighing 225–250 g were obtained from Charles River Laboratories (Canada). Throughout the experiment, they were pair-housed (12:12 light-dark cycle, with ambient temperature of 21 ± 2 °C, relative humidity of 50–5%) and given free access to water and food ad libitum. Shelter and nesting material were used as environment enrichments.

2.2. Amphetamine-induced sensitized state

One week after arrival, d-amphetamine (Sigma A-5880) was administered via intraperitoneal route (*i.p.*) three times a week (i.e. Monday, Wednesday, and Friday) for five consecutive weeks as previously described [27]. On week 1, animals received a dose of 1 mg/kg (from amphetamine salt), and the dose was increased by 1 mg/kg each week. By the final week (Week 5), animals were given amphetamine at a dose of 5 mg/kg. Control animals received saline under the same injection schedule. To confirm the amphetamine-induced sensitized state, all animals were given a challenge injection of amphetamine (0.5 mg/kg) on Week 6, and their locomotor activities were recorded for 1 h [23,24]. All injections were administered at a 1 ml/kg volume 1 h after the amphetamine challenge and the rats were killed by decapitation under isoflurane anesthesia. The ventral and dorsal striatum were dissected out and frozen on dry ice.

2.3. Locomotor activity

Following the five-week schedule of amphetamine sensitization, animals were placed in Plexiglas cages (27 × 48 × 20 cm) for 1 h daily, for three consecutive days to habituate them to the testing equipment. On the experiment day, animals were placed in the same Plexiglas cages for 1 h to record their baseline locomotor activity, followed by an acute amphetamine challenge. Immediately after the challenge injection, animals were returned to the Plexiglas cages for another hour of recording. Each Plexiglas cage was equipped with six photo-beam cells to detect horizontal movement.

2.4. Membrane preparation

The tissue was placed in 10 ml of 50 mM Tris-HCl, pH 7.4, with 7 mM $MgCl_2$, 1 mM EDTA and a cocktail of protease inhibitors (Roche Diagnostics, Mannheim, Germany). The tissue was homogenized using a sonicator (Soniprer 150). Samples were sonicated 15 s and then kept for 30 s on ice for at least three times until they were subjected to centrifugation at 4 °C for 45 min at 40,000 ×*g* (Thermo scientific, Sorvall Lynx 6000, Stockholm, Sweden). The supernatant was discarded and the membrane pellet was resuspended by sonication in the same buffer and centrifuged using the same conditions as before. The protein concentration was determined for the membrane homogenates by means of BCA Protein Assay (Pierce, Sweden) using bovine serum albumin (BSA) as standard. Pelleted membranes were resuspended to a concentration of 2 mg/ml, immediately used or stored at − 80 °C until required.

2.5. [^{3}H]-Raclopride binding experiments

[^{3}H]-raclopride binding was displaced by quinpirole to determine Ki_H and Ki_L values for the agonist binding sites from the competition curves obtained from each rat. Briefly, striatal rat membrane preparations (100 μg protein/ml) were incubated with increasing concentrations of quinpirole (ranging from 0.3 nM–3 mM) and 2 nM [^{3}H]-raclopride (specific activity 78.1 Ci/mmol, PerkinElmer Life Sciences, Stockholm, Sweden) in 250 μl of IB (50 mM Tris-HCl, 100 mM NaCl, 7 mM $MgCl_2$, 1 mM EDTA, 0.05% BSA, 1 mM DTT) for 90 min at 30 °C, in the presence or absence of 100 nM of the A2A 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 Whatman GF/B filters (Maidstone, Kent, UK) using a MultiScreen™ Vacuum Manifold 96-well (Millipore Corp, Bedford, MA), followed by three washes (~250 μl per wash) with ice-cold washing buffer (50 mM Tris-HCl pH 7.4). The filters were dried, 5 ml of scintillation cocktail was added and the bound ligand was determined after 12 h by liquid scintillation spectrometry.

2.6. Statistical analysis

The number of rats (n) in each experimental condition is indicated in figure legends. Data from competition experiments were analyzed by nonlinear regression analysis using GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA). Ki_H and Ki_L values from several replications in each rat are given as means ± SEM. The effects of CGS21680 on these values and on the proportion of D2R like agonist binding sites in the high affinity state (RH) were evaluated with paired Student's *t*-test and Mann Whiney *U* test, respectively. The percent change induced by CGS 21680 in the Ki_H and Ki_L values in the amphetamine sensitized state vs the saline sensitized state was compared using the Mann-Whitney *U* test. The behavioral assessment was analyzed by two-way ANOVA, followed by Tukey's post hoc analysis (IBM SPSS Statistics). The p value 0.05 and lower was considered significant.

3. Results

3.1. Amphetamine-induced locomotor activity in amphetamine sensitized rats

The baseline locomotor activity was not statistically different between the amphetamine-sensitized group and the vehicle control group (Tukey's post hoc analysis; $p > 0.05$). After the acute challenge of amphetamine, however, the amphetamine-sensitized group exhibited a higher level of locomotor activity compared to the saline sensitized control group (two-way ANOVA; F3, 27 = 18.376; $p < 0.001$) (Fig. 1). Meanwhile, the saline sensitized control group displayed no difference in its locomotor activity from its baseline ($p > 0.05$).

3.2. ^{3}H-Raclopride/quinpirole competition experiments. Effects of CGS 21680 in membrane preparation from the ventral striatum

3.2.1. Saline sensitized rats after acute amphetamine challenge (0.5 mg/kg)

As seen in Table 1 and Fig. 2, the adenosine A2AR agonist CGS 21680 (100 nM) added to the membrane preparations failed to significantly alter the pKi_H and pKi_L values obtained from the competition curves with the D2R like agonist quinpirole. Also the proportion of D2Rs in the high affinity state (RH values) was not changed by CGS 21680.

3.2.2. Amphetamine sensitized rats after acute amphetamine challenge (0.5 mg/kg)

The pKi_H and pKi_L values were not significantly altered by CGS 21680 nor was the proportion of D2Rs in the high affinity state (Table 1) in spite of an indicated shift to the right in the competition curve in the