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# The role of 5-$HT_{2A}$ receptor antagonism in amphetamine-induced inhibition of A10 dopamine neurons in vitro

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**Abstract**

The role of the 5-$HT_{2A}$ receptor in modulating amphetamine-induced inhibition of dopamine neuronal firing in A9 and A10 was investigated in rat midbrain slices. The antipsychotic drugs olanzapine and clozapine more potently reversed the amphetamine-induced inhibition in A10 neurons compared to A9 neurons. Risperidone (0.03 and 0.1 μM) reversed amphetamine-induced inhibition of firing activity similarly in A9 and A10. The dopamine D2 receptor antagonist (−)sulpiride (0.05 and 1 μM) reversed the amphetamine (10 μM)-induced inhibition of firing activity in A9 and A10 neurons. The selective 5-$HT_{2A}$ receptor antagonist MDL100907 (0.05 μM), strongly enhanced the reversal of amphetamine-induced inhibition by (−)sulpiride in A10, but its effectiveness was much smaller in A9 dopamine neurons.

We conclude that 5-$HT_{2A}$ receptor antagonism enhanced reversal of amphetamine-induced inhibition by dopamine D2 antagonism in A10, suggesting that dopamine $D_2$ receptor antagonism combined with 5-$HT_{2A}$ receptor antagonism may play a role in antipsychotic drug atypicality.


*Keywords:* Substantia nigra; Ventral tegmental area; Serotonin; Extracellular recording; Antipsychotic drug

## 1. Introduction

Increased activity of the mesoaccumbal dopamine circuitry originating in the ventral tegmental area (A10) has been hypothesized to underlie some of the symptoms of schizophrenia (Arnt and Skarsfeldt, 1998; Pani, 2002). Antipsychotic drugs used to treat schizophrenia also suppress the nigrostriatal system originating in the substantia nigra pars compacta (A9) and have been implicated in extrapyramidal side-effects (Crocker and Hemsley, 2001; Grace et al., 1997; Wong and Van Tol, 2003). The more recently developed atypical antipsychotic drugs such as clozapine and olanzapine have a broader range of receptor affinities and produce less extrapyramidal side-effects compared to the classical antipsychotic drugs (Conley and Kelly, 2002; Grace et al., 1997; Kapur and Remington, 2001; Tarsy et al., 2002). Consequently, much effort has been invested in the development of new antipsychotic drugs that only possess therapeutic efficacy without the unwanted extrapyramidal side-effects. The main approach has been to specifically target the A10 system, avoiding effects on the A9 system.

Amphetamine-induced inhibition of dopamine neurons is used as an in vivo model to determine potential antipsychotic activity (Ellenbroek and Cools, 2000; Stockton and Rasmussen, 1996). Amphetamine, a potent central nervous system stimulant drug with psychotomimetic properties, elevates extracellular dopamine by promoting non-vesicular dopamine release (via the dopamine transporter) and blocking dopamine re-uptake (Byrnes and Wallace, 1997; Jones et al., 1999; Sulzer et al., 1995). The increased extracellular dopamine level inhibits dopamine neuron firing activity via dopamine D2 auto receptors. Reversal of amphetamine-induced inhibition of dopamine neurons is a common property of clinically effective antipsychotic drugs (Bunney et al., 1973; Goldstein et al.,

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1993; Stockton and Rasmussen, 1996; White and Wang, 1983). This property is used as a test to predict therapeutic efficacy (reversal in A10) and extrapyramidal side-effects liability (reversal in A9). In this model classical antipsychotic drugs reverse amphetamine-induced inhibition in both A9 and A10 dopamine neurons, while atypical antipsychotic drugs tend to reverse amphetamine-induced inhibition more potently in A10 than in A9 dopamine neurons. Reversal of amphetamine-induced inhibition by antipsychotic drugs is explained by their dopamine D2 receptor antagonistic properties. However, it is unclear why atypical antipsychotic drugs reverse amphetamine-induced inhibition more potently in A10 dopamine neurons. Atypical antipsychotic drugs also have affinity for the 5-$HT_{2A}$ receptor (Arnt and Skarsfeldt, 1998), therefore it is hypothesized that this receptor is a contributing factor in the observed difference in reversal. This is supported by the observations that 5-$HT_{2A}$ receptor activation can modulate the activity of A10 dopamine neurons (Brodie and Bunney, 1996; Doherty and Pickel, 2000; Olijslagers et al., 2004; Sorensen et al., 1992), while such a role was not observed in A9 dopamine neurons.

In this study we determined if 5-$HT_{2A}$ receptor antagonism could affect reversal of amphetamine-induced inhibition by a dopamine $D_2$ receptor antagonist in vitro in a rat midbrain slice preparation.

## 2. Material and methods

### 2.1. Preparation of brain slices

After decapitation (male Wistar rats; 75–100 g; Harlan, Zeist, The Netherlands) the brain was quickly removed and placed in ice-cold artificial cerebrospinal fluid (aCSF) of the following composition (in mM): NaCl 120, KCl 3.5, $MgSO_4$ 1.2, $NaH_2PO_4$ 1.25, $CaCl_2$ 2.5, D-glucose 10, $NaHCO_3$ 25, ascorbic acid 1 and gassed with a mixture of 95% $O_2$ and 5% $CO_2$. A tissue block was prepared from the brain and coronal slices (350 μm thick) containing both the A9 and the A10 region were cut in ice-cold aCSF with a vibratome (model VT1000S, Leica). Immediately after cutting, the slice was transferred to warm aCSF (35 °C) for 20 min, where after it was stored at room temperature for later use (Werkman et al., 2001). For recording, the slice was transferred to a recording chamber (volume ≈ 1 ml), which was continuously perfused with aCSF (≈2 ml/min) and held at 35 °C. After approximately 30 min of equilibration, extracellular recordings of dopamine neurons in the A9 and the A10 region started.

### 2.2. Extracellular recordings

Extracellular recordings were made with electrodes that were pulled with a micropipette puller (Brown/Flaming P-87; Sutter Instruments, CA, USA) from thin-wall borosilicate glass pipettes (1.5 mm outer diameter, Science Products, Hofheim, Germany) and filled with aCSF. One electrode was placed in A9 and another one in A10 at positions where they each recorded a spontaneously active dopamine neuron. The following criteria had to be fulfilled before a neuron was considered dopaminergic (Olijslagers et al., 2004; Werkman et al., 2001): (i) a regular firing pattern (0.5–8 Hz); (ii) a broad (>2 ms), triphasic action potential; and (iii) quinpirole sensitivity to a concentration below 0.3 μM, resulting in cessation of action potential firing. Neurons in A9 and A10 that fulfill the electrophysiological criteria listed above have previously been identified by immunocytochemistry as dopamine-containing (Grace and Onn, 1989). The extracellular signals were high-pass filtered at 300 Hz and digitized at 4 kHz with an ADC converter under control of a personal computer for off-line analysis.

### 2.3. Drugs

Unless otherwise mentioned drugs were obtained from Solvay Pharmaceuticals (Weesp, The Netherlands). Stock solutions were prepared as follows: 10 mM (−)sulpiride, 10 mM clozapine, 10 mM olanzapine, 10 mM risperidone in 0.01 M HCl. MDL100907 (4-piperidinemethanol) stock (5 mM) was made in dimethylsulfoxide (DMSO). Quinpirole (10 mM, Sigma, St. Lois, MO, USA) and amphetamine (1 mM, Duchefa Farma BV, Haarlem, Netherlands) stock solutions were made in $H_2O$. The solutions were diluted to the final concentrations in aCSF and applied to the slices by superfusion. DMSO concentration never exceeded 0.01%.

### 2.4. Data analysis

An analysis program running on the personal computer detected action potentials by means of template matching. This emphasized the characteristic shape of the extracellular action potentials of the dopamine neurons and allowed quantification of the neuronal activity even if gradual variations in extracellular action potential amplitude occurred. The times of occurrence of action potentials during control periods and during drug applications were determined and used to calculate the firing rate (spikes/s) in bins of 5 s. The mean baseline firing rate of each neuron was determined for at least 2–3 min. Amphetamine-induced reduction of the firing rate in respect to the baseline level was calculated for each neuron. The $IC_{50}$ values were calculated using the fits to the logistic equation of the form:

$$F(C) = \frac{F_0}{1 + (C/\mathrm{IC}_{50})^h}$$

in which $F(C)$ is the firing rate at concentration $C$, $F_0$ the mean baseline firing rate, $IC_{50}$ the concentration that induces