Neuropharmacology 82 (2014) 69–75

Contents lists available at ScienceDirect

Neuropharmacology

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

The involvement of accumbal glycine receptors in the dopamine-elevating effects of addictive drugs

Susanne Jonsson [a,*], Louise Adermark [a], Mia Ericson [a], Bo Söderpalm [a,b]

[a] Addiction Biology Unit, Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, The Sahlgrenska Academy, University of Gothenburg, Medicinaregatan 11, PO Box 410, SE 405 30 Gothenburg, Sweden
[b] Beroendekliniken, Sahlgrenska University Hospital, Gothenburg, Sweden

ARTICLE INFO

Article history:
Received 12 November 2013
Received in revised form
14 March 2014
Accepted 18 March 2014
Available online 29 March 2014

Keywords:
Addictive drugs
Dopamine
Glycine receptors
Microdialysis
Nucleus accumbens

ABSTRACT

The ability of drugs of abuse to increase mesolimbic levels of dopamine is a characteristic associated with their rewarding effects. Exactly how these effects are produced by different substances is not as well characterised. Our previous work in rats has demonstrated that accumbal glycine receptors (GlyRs) are involved in mediating the dopamine-activating effects of ethanol, and in modulating ethanol intake. In this study the investigation of GlyR involvement was extended to include several different drugs of abuse. By using microdialysis and electrophysiology we compared effects of addictive drugs, with and without the GlyR antagonist strychnine, on dopamine levels and neurotransmission in nucleus accumbens. The dopamine-increasing effect of systemic ethanol and the drug-induced change in neurotransmission *in vitro*, as measured by microdialysis and field potential recordings, were dependent on GlyRs in nAc. Accumbal GlyRs were also involved in the actions of tetrahydrocannabinol and nicotine, but not in those of cocaine or morphine. These data indicate that accumbal GlyRs play a key role in ethanol-induced dopamine activation and contribute also to that of cannabinoids and nicotine.

1. Introduction

Drug addiction is a serious socioeconomic problem worldwide and studies have shown that initial reactions to drugs of abuse may predict future use (de Wit and Phillips, 2012). A common effect of addictive drugs is that they increase extracellular dopamine (DA) levels in the mesolimbic dopamine system (Di Chiara, 2000; Di Chiara and Imperato, 1988), which in the nucleus accumbens (nAc) has been associated with both drug "liking" (Boileau et al., 2003; Drevets et al., 2001) and drug "wanting" (Leyton et al., 2002; Wyvell and Berridge, 2000). Either way, this dopamine effect is believed to create motivation to repeat consumption of the drug, which eventually may lead to dependence and addiction.

Although ethanol is one of the addictive drugs most widely used, and abused, the mechanisms by which it exerts its rewarding effects are unclear and disputed. Our previous investigations have demonstrated that local blockade of accumbal glycine receptors (GlyRs) prevents the DA-activating effects of ethanol (Soderpalm et al., 2000, 2009). Local administration of strychnine (a selective and competitive GlyR antagonist) concentration-dependently reduced both basal DA levels in the nAc and the DA releasing effect of local ethanol (Molander and Soderpalm, 2005a,b). In addition, the reduced ethanol consumption in rats following administration of glycine re-uptake inhibitors also supports a role for GlyRs in mechanisms related to ethanol reinforcement (Lido et al., 2012; Molander et al., 2007; Vengeliene et al., 2010).

After GABA the glycinergic system is the second most important inhibitory neurotransmitter system in the CNS. Besides its specific strychnine-sensitive receptor, glycine is also a co-agonist at the N-Methyl-D-aspartate (NMDA) receptor (Curtis et al., 1968; Young and Snyder, 1973). In the mammalian CNS GlyRs are most abundant in the spinal cord, brainstem and the cerebellum where they are involved in essential functions such as respiration, sensory processing and motor control (Betz and Becker, 1988; Yevenes and Zeilhofer, 2011). Structurally the GlyR belongs to the pentameric cys-loop superfamily of ligand-gated ion channels and exists as either α-homomers or αβ-heteromers, with a stoichiometry of 2α3β (Grudzinska et al., 2005; Lynch, 2009). Subunit composition seems to affect localisation as heteromeric GlyRs are found in the synapse whereas homomers are found extra-synaptically (Deleuze et al., 2005). Apart from glycine, taurine and β-alanine are also endogenous ligands at the GlyR.

* Corresponding author. Tel.: +46 31 786 3975.
E-mail address: Susanne.Jonsson@neuro.gu.se (S. Jonsson).

http://dx.doi.org/10.1016/j.neuropharm.2014.03.010
0028-3908/

Since drugs of abuse share the ability to increase accumbal DA they may possibly share features/components leading up to this effect. The involvement of GlyRs in ethanol's DA effect has repeatedly been illustrated, but to our knowledge not studied in reference to other addictive drugs. Thus, the aim of this study was to investigate whether the GlyR involvement is a specific feature for ethanol, or if accumbal GlyRs are involved in mediating the DA-activating effects also of other drugs of abuse. Microdialysis and electrophysiology were used to investigate different aspects of potential GlyR involvement in mediating acute effects of various addictive drugs (including ethanol) on neurotransmission in the nAc.

2. Materials and methods

2.1. Animals

In this experiment male Wistar rats (Taconic, Denmark), weighing 270–320 g, were used and kept under regular light–dark conditions (lights on at 7.00 AM and off at 7.00 PM). Experiments began one week after arrival, allowing the animals to adapt to the facilities (20–22 °C and a humidity of 50–65%). The animals had access to standard rat feed (Lantmännen, Stockholm, Sweden) and tap water *ad libitum*. All experiments were carried out in accordance with the European Communities Council Directive (86/609/EEC), and approved by the Ethics Committee for Animal Experiments, Gothenburg, Sweden.

2.2. Drugs

The substances chosen for this study included representatives of the cannabinoid, psychostimulant and opiate drug classes, in addition to ethanol and nicotine. Ethanol (95% Kemetyl AB, Haninge, Sweden) was dissolved in physiological saline solution (0.9%) to a concentration of 15% and administered i.p. at a dose of 2.5 g/kg. Cocaine and morphine (Apoteket AB, Stockholm, Sweden) were dissolved in physiological saline solution (0.9%) and doses of 15 mg/kg and 10 mg/kg (cocaine) and 5 mg/kg (morphine), were administered i.p. at a volume of 2 ml/kg. Nicotine hydrogen tartrate salt (Sigma–Aldrich, Stockholm, Sweden), was dissolved in 0.9% saline and then neutralised using sodium bicarbonate before injected s.c. (2 ml/kg) at a dose of 0.4 mg/kg. The nicotine dose is expressed as free base. Δ^9-Tetrahydrocannabinol (THC) is the component primarily inducing the psychoactive effects of cannabis. THC (Sigma–Aldrich, Stockholm, Sweden) 25 mg/ml in ethanol was dissolved in 45% w/v 2-hydroxypropyl-β-cyclodextrin (β-cyclodextrin, Sigma–Aldrich) and a dose of 3 mg/kg was administered i.p. at a volume of 2 ml/kg. The drug doses used have been shown to elicit robust increases in DA levels (Cadoni and Di Chiara, 1999; Ericson et al., 2011, 2009; Kalivas and Duffy, 1993; Malone and Taylor, 1999). For electrophysiological experiments THC was dissolved in β-cyclodextrin before diluted to its final concentration, 1 μM, in artificial cerebrospinal fluid containing (in mM); 124 NaCl, 4.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 26 $NaHCO_3$, 1.2 NaH_2PO_4 and 10 D-glucose, continuously bubbled with a mixture of 95% O_2/5% CO_2 gas (Adermark et al., 2011a). Strychnine (Sigma–Aldrich, Stockholm, Sweden) was dissolved in Ringer solution to a concentration of 20 μM and administered in the nAc via reversed microdialysis. Ringer solution consisted of (in mmol/l): 140 NaCl, 1.2 $CaCl_2$, 3.0 KCl, and 1.0 $MgCl_2$.

2.3. Microdialysis

Two days prior to the experiment the dialysis probes were surgically implanted, as previously described (Lido et al., 2009). In short, rats were anaesthetised with isoflurane and mounted into a stereotaxic instrument. Holes were drilled for the placement of two anchoring screws and for an I-shaped dialysis probe (custom-made in the laboratory). The dialysis probe was lowered into the nAc (A/P: +1.85, M/L: −1.4 mm relative to bregma, V/D: −7.8 mm relative to dura; Paxinos and Watson, 2007). The exposed length of the dialysis membrane was 2 mm and the nAc dialysis probes were placed in the core–shell border region. After positioning the probes and the anchoring screws they were fixed to the skull with Harvard cement (DAB Dental AB, Gothenburg, Sweden). After two days of recovery the dialysis experiment was performed. The sealed inlet and outlet were cut open and connected to a microperfusion pump (U-864 Syringe Pump AgnTho's, Lidingö, Sweden) via a swivel, allowing the animal to move around freely. The probes were perfused with Ringer solution at a rate of 2 μl/min and dialysate samples (40 μl) were collected every 20 min. To obtain a balanced fluid exchange the rats were perfused with Ringer solution for 2 h before baseline sampling began. Animals were killed directly after the experiment, brains were removed and probe placements verified (Fig. 1A). Treatment with strychnine was used to examine the potential effect of GlyR blockade on the DA increasing effect of the different drugs examined in this study. Following baseline sampling strychnine perfusion in the nAc started (and continued throughout the experiment) and 40 min later (at $t = 40$) the drugs were administered systemically via an i.p. or s.c. injection. For each addictive drug at least four perfusion/injection treatments were used; Ringer/drug, strychnine/drug, strychnine/saline, and Ringer/saline.

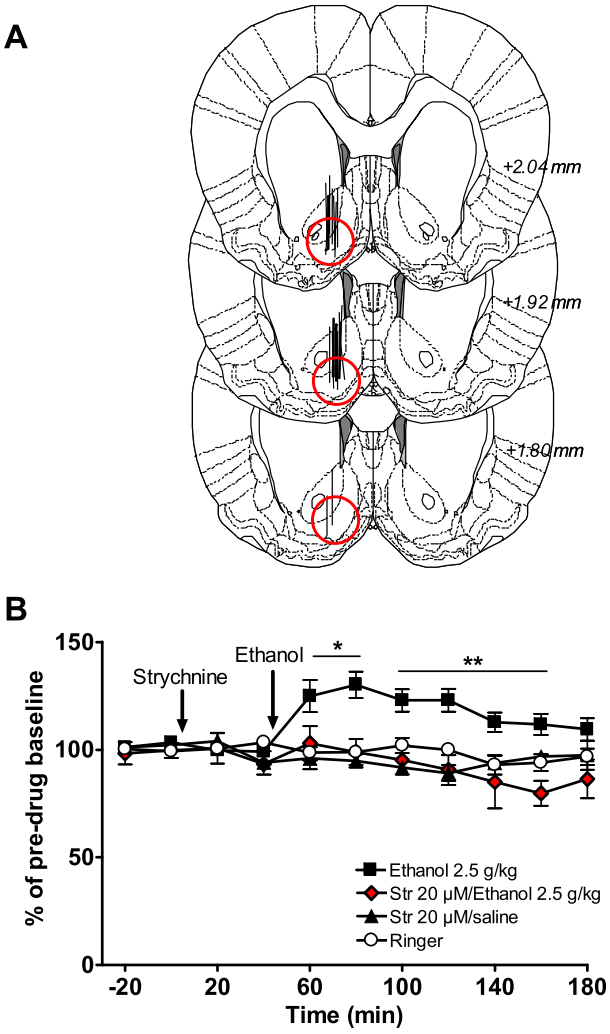

Fig. 1. The effect of strychnine on ethanol's dopamine-increasing effect in the nucleus accumbens, measured with *in vivo* microdialysis. (A) A, for the study, representative sample of verified probe traces in nucleus accumbens (nAc) is shown in this figure adapted from Paxinos and Watson (2007). (B) The effects of different treatments on dopamine (DA) levels in the nAc were investigated using *in vivo* microdialysis. Local perfusion with strychnine (20 μM), starting 40 min before systemic drug administration, abolished the ethanol-induced DA increase in this region but did not affect DA levels *per se*. Arrows indicate the start of drug administration. Results are presented as means ± SEM, $n = 5–12$ (after observing a stable effect in all strychnine/ethanol-treated rats a group of 5 animals was considered adequate). Only significant differences between ethanol and strychnine/ethanol animals are presented, $^{*}p < 0.05$, $^{**}p < 0.01$.

2.4. HPLC analysis of dopamine

To analyse the concentrations of dopamine in the dialysate samples, a high-pressure liquid chromatography (HPLC) system was used for the separation and detection of dopamine in accordance with previous description (Lido et al., 2009). An external standard, containing 3.25 fmol/μl of dopamine, was used to identify the dopamine peak. Four baseline samples, collected prior to any treatment, were considered 100% and all of the following samples were calculated as % of change from baseline.

2.5. Electrophysiology

Field potential recordings were performed as previously described (Adermark et al., 2011a), using 400 μM thick coronal brain slices prepared from drug-naïve adult Wistar rats. Since ethanol affects striatal activity through modulation of GABAergic neurotransmission $GABA_A$ receptor inhibitors were omitted in all recordings (Adermark et al., 2011c; Mishra et al., 2012). Recorded population spikes (PS) were activated by a monopolar stimulation electrode located in the nAc shell with a 20 s interval. Administration of strychnine (1 μM) has previously been shown to induce a small but significant increase in PS amplitude in the nAc (Adermark et al.,