Neuropharmacology 62 (2012) 1834–1840

Contents lists available at SciVerse ScienceDirect

Neuropharmacology

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

Modifications of the input currents on VTA dopamine neurons following acute versus chronic cocaine exposure

Avner Michaeli, Henry Matzner, Tatyana Poltyrev, Rami Yaka*

Institute for Drug Research, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel

ARTICLE INFO

Article history:
Received 21 July 2011
Received in revised form
30 November 2011
Accepted 7 December 2011

Keywords:
AMPA receptor
$GABA_A$ receptor
NMDA receptor
Ratio
Picrotoxin
ePSC

ABSTRACT

Excitatory synapses on dopamine (DA) neurons in the ventral tegmental area (VTA) are modulated following exposure to various addictive drugs, including cocaine. Previously we have shown that cocaine affects $GABA_A$ receptor ($GABA_AR$)-mediated neurotransmission in VTA DA neurons. This finding led us to reexamine the modulation of the excitatory synapse on these neurons in response to cocaine exposure, while the activity of $GABA_AR$ is uninterrupted. Using rat brain slices, evoked post synaptic currents (ePSC) were monitored and inhibitors of NMDA receptor (NMDAR) and AMPA receptor (AMPAR) were gradually added to inhibitors-free bath solution. Modifications in the efficacy of the excitatory synapses were evaluated by comparing AMPAR-mediated and NMDAR-mediated currents (AMPA/NMDA ratio). The lack of $GABA_AR$ inhibitors enabled us to examine parallel changes in the relation between $GABA_AR$-mediated and NMDAR-mediated currents ($GABA_A$/NMDA ratio). First, we found that AMPA/NMDA ratio measured under complete availability of $GABA_AR$, is significantly higher than the ratio measured under $GABA_AR$ blockade. In addition, $GABA_A$/NMDA ratio, but not AMPA/NMDA ratio, is augmented a few hours following *in vitro* acute cocaine exposure. When measured 24 h after *in vivo* single cocaine injection, no change in $GABA_A$/NMDA ratio was observed, however, the AMPA/NMDA ratio was found to be significantly higher. Finally, a decrease in both ratios was detected in rats repeatedly injected with cocaine.

Taken together, these results lead to a better understanding of the means by which cocaine modifies synaptic inputs on VTA DA neurons. The parallel changes in $GABA_A$/NMDA ratio may suggest an interaction between inhibitory and excitatory neural systems.

1. Introduction

A distinctive approach has previously been suggested to assess plasticity of excitatory synapses – calculating the relative contribution of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPAR)-mediated and *N*-methyl-D-aspartate receptor (NMDAR)-mediated currents to the total excitatory current (Thomas et al., 2001; Ungless et al., 2001). Thus, a change in this ratio (AMPA/NMDA ratio) is regarded as an indicator for alteration in synaptic strength. Among various circumstances, exposure to a number of addictive drugs including cocaine has been demonstrated to cause an increase in AMPA/NMDA ratio in dopamine (DA) neurons in the ventral tegmental area (VTA) (Saal et al., 2003; Ungless et al., 2001). Typically, recording of AMPA/NMDA ratio is performed in the presence of blocker of γ-amino-butyric acid receptor type A ($GABA_AR$)-mediated currents, the most widespread inhibitory current in the brain. Recently we and other have found that cocaine exposure modulates $GABA_AR$ currents in VTA DA neurons (Michaeli and Yaka, 2010, 2011; Pan et al., 2008; Steffensen et al., 2008). Therefore, evaluating the manner by which cocaine modulates the properties of VTA synapses turns to be more adequate while the activity of $GABA_AR$ is uninterrupted.

Previously it was shown that following repeated, but not single, *in vivo* cocaine injections the excitatory synapses on VTA DA neurons become susceptible to develop long term potentiation (LTP), using spike timing pairing (STP) protocol. No potentiation could be detected after either single or repeated saline injections. In addition, it was suggested that this compliance to develop LTP was feasible due to cocaine-induced reduction in $GABA_A$ neurotransmission in VTA DA neurons (Liu et al., 2005). On the other hand,

Abbreviations: AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor; APV, D-(−)-2-amino-5-phosphonopentanoic acid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; DA, dopamine; ePSC, evoked post synaptic currents; $GABA_AR$, γ-amino-butyric acid receptor type A; LTP, long term potentiation; NMDAR, *N*-methyl-D-aspartate receptor; SDP, steady depolarization pairing; STP, spike timing pairing; VTA, ventral tegmental area.

* Corresponding author. Department of Pharmacology, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, POB 12065, Jerusalem 91120, Israel. Tel.: +972 2 6758735; fax: +972 2 6758420.
E-mail address: yaka@md.huji.ac.il (R. Yaka).

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doi:10.1016/j.neuropharm.2011.12.005

earlier study has shown that induction of LTP, using steady depolarization pairing (SDP) protocol, of the excitatory synapses on VTA DA neurons is obtainable following *in vivo* saline injection. However, single cocaine injection potentiates the excitatory synapses by itself, and LTP can not be induced following such treatment since the potentiation of the excitatory synapses is already saturated (Ungless et al., 2001). The discrepancy between the seemingly contradictory findings was partially attributed to the different LTP induction protocols used in each study (Liu et al., 2005). A newer study examined the induction of LTP of excitatory synapses in VTA DA neurons, using STP protocol similar to Liu et al. following single and repeated saline or cocaine injections. In contrast to the findings of Liu et al. LTP could be induced in slices taken from saline-treated rats, but not from single, as well as repeated, cocaine-treated rats (Argilli et al., 2008). Interestingly, the recording conditions in Liu et al. study were significantly different from those in the two other studies, and a major difference was an absence or the presence of $GABA_A$R blocker, respectively.

In the current study, we aimed to test the hypothesis that the activity of $GABA_A$R affects the responsiveness of excitatory synapses to cocaine exposure. Thus, the effect of cocaine on AMPA/NMDA ratio was determined, while $GABA_A$ neurotransmission remained unblocked. Moreover, parallel changes in the ratio between $GABA_A$R and NMDAR currents ($GABA_A$/NMDA ratio) were examined as well.

2. Material and methods

2.1. Animals

The experiments were conducted according to the guidelines of the Institutional Animal Care Committee of the Hebrew University (Jerusalem, Israel). All the animals used in this study were 18–26 days old male Sabra rats, a strain of the Hebrew University.

2.2. Slice preparation

Rats (Harlan Biotech, Jerusalem) were decapitated under isoflurane anesthesia and tissue block containing the midbrain was rapidly isolated. Horizontal slices, 250 μm thick, were prepared in ice-cold low-calcium artificial cerebrospinal fluid (aCSF) solution containing (in mM): 126 NaCl, 1.6 KCl, 1.2 NaH_2PO_4, 1.2 $MgCl_2$, 18 $NaHCO_3$, 11 glucose, and 0.625 $CaCl_2$, using a vibratome VT1000S (Leica, Nussloch, Germany). Slices were transferred to a holding chamber filled with the same solution, with the exception of the $CaCl_2$ concentration, which was changed to 2.4 mM (32–34 °C). Slices were allowed to recover for at least 1 h before being placed in a recording chamber where the last solution continuously flows (2 ml/min). All solutions were saturated constantly with 95% O_2 and 5% CO_2 throughout the experiments.

2.3. Electrophysiology

Neurons were visualized using an upright microscope with infrared illumination. Whole-cell currents were recorded in a voltage-clamp configuration using a Multiclamp 700B amplifier (Axon Instruments, Foster City, CA). Electrodes pulled from glass capillaries (3–6 MΩ resistance) were filled with an internal solution containing the following (in mM): 117 $CsCH_3SO_3$, 20 HEPES, 0.4 EGTA, 2.8 NaCl, 5 TEA-Cl, 2.5 Mg_2ATP, and 0.3 Na_3GTP, pH 7.2–7.4. The recordings were performed at a holding potential of +40 mV, unless otherwise is specified. Series and input resistance were monitored continuously with a 4 mV depolarizing step given before every afferent stimulus and experiments were begun only after series resistance had stabilized. Data were discarded if series resistance changed by more than 15%. DA neurons were identified by the presence of Ih-currents (Johnson and North, 1992) tested immediately after break-in, using a series of 8 successive hyperpolarizing steps (−10 mV; 250 ms) beginning from an initial holding potential of −50 mV. The presence of Ih-currents does not unequivocally correlate with tyrosine-hydroxylase positive neurons. However, since it characterizes most of VTA DA neurons (Sarti et al., 2007) but not non-DA neurons (Margolis et al., 2006), the results presented in the current study are likely characterize DA neurons. For evoked post synaptic currents (ePSC) recording, a bipolar stainless steel stimulating electrode was placed 100 μm rostral to the recording electrode and was used to stimulate afferent fibers at a frequency of 0.1 Hz. Slices used for recording were discarded after each experimental procedure. Data were filtered at 2 kHz, digitized at 10 kHz, and collected online. Recording of ePSC was performed using acquisition software Igor Pro (WaveMetrics, Lake Oswego, OR). Recording of Ih-currents was performed using the acquisition software pClamp 10 (Molecular Devices, Foster City, CA). All chemicals were purchased from Sigma–Aldrich (St. Louis, MO), except APV (D-(−)-2-Amino-5-phosphonopentanoic acid; 50 μM), CNQX (6-Cyano-7-nitroquinoxaline-2,3-dione; 10 μM) and picrotoxin, which were purchased from Tocris Bioscience (Bristol, UK).

2.4. Data analysis

Calculating the specific currents, that form the complete ePSC, was performed using Igor Pro, as described in the results chapter. Unless otherwise specified, the amplitude of the evoked currents was calculated by measuring the difference between the peak of the current and the baseline current immediately before the stimulus artifact. The changes in the amplitude of evoked currents, shown by time-course graphs, were calculated by comparing the averaged amplitude at 35–40 min with that at 5–10 min. Sample traces shown in the figures represent an average of 10–16 traces. Data sets were compared using *t* test or paired *t* test at a significance level of 0.05. All values are presented as average ± SEM.

3. Results

3.1. Isolating specific receptor-mediated evoked current from the total evoked current

Previous studies, examined the ratio between AMPAR-mediated and NMDAR-mediated currents, were conducted under a general blockade of $GABA_A$R-mediated currents, in order to isolate the relevant excitatory currents. Since we were interested to examine this ratio without affecting $GABA_A$ neurotransmission, no blocker of $GABA_A$R was included during the recordings. The recordings at this series of experiments were performed as follows: a selected putative DA neuron was clamped to +40 mV to allow development of NMDAR currents in addition to the available AMPAR and $GABA_A$R currents. At this membrane potential, using cesium-based internal solution (see Methods), all three currents flow outwardly. After stabilization of the total ePSC the NMDAR blocker, APV, was added to the bath solution, and the remaining ePSC (AMPAR and $GABA_A$R currents) was recorded for additional 8 min. Then, the AMPAR blocker, CNQX, was added to the bath solution for additional 8 min (Fig. 1*a*). In the presence of APV and CNQX, the ePSC represents $GABA_A$R current, which is completely blocked by 100 μM picrotoxin (Fig. 1*c*). AMPAR current is calculated by digital subtraction of $GABA_A$R current from the ePSC recorded in the presence of APV alone. NMDAR current is calculated by subtracting the ePSC recorded in the presence of APV alone from the original ePSC (Fig. 1*b*). Accordingly, this method allows differentially evaluating the contribution of each current to the total evoked current.

3.2. The presence of picrotoxin alters the AMPA/NMDA ratio, recorded from DA neurons of naïve VTA slices

First, the possibility that blockade of $GABA_A$R currents affects the AMPA/NMDA ratio was tested. VTA slices of naïve rat were taken for recording. A half of the recordings from each rat ($N = 7$) was conducted in the presence of picrotoxin, as previously described (Ungless et al., 2001), while the other half was conducted in the absence of any $GABA_A$R blocker, as described above. The mean AMPA/NMDA ratio, measured from DA neurons, was found to be significantly higher when picrotoxin was not included during the recording (picrotoxin presence, 0.43 ± 0.4; picrotoxin absence, 0.92 ± 0.12; $n = 12$ for each group; $p < 0.001$) (Fig. 2*a*, *b*). The only difference between the two treatments is the presence of picrotoxin at the bath solution during the recordings, which usually begin less than 1 h after placing the brain slice in the recording chamber. This means that the short continuous exposure to picrotoxin either decreases AMPAR currents or increases NMDAR currents or both. In order to find the manner by which picrotoxin by itself reduces AMPA/NMDA ratio, AMPAR and NMDAR evoked currents were recorded before and 30 min following continuous picrotoxin exposure. By using the same