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Dopamine-related drugs act presynaptically to potentiate $GABA_A$ receptor currents in VTA dopamine neurons

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ABSTRACT

Electrical activity of ventral tegmental area (VTA) dopamine (DA) neurons is immediately inhibited following *in vivo* administration of cocaine and other DA-related drugs. While various forms of synaptic modulation were demonstrated in the VTA following exposure to DA-related drugs, comprehensive understanding of their ability to inhibit the activity of DA neurons, however, is still lacking. In this study, using whole-cell patch-clamp recordings from rat brain slices, a novel form of synaptic modulation induced by DA-related drugs was isolated. DA exposure was shown to cause potentiation of γ-amino-butyric acid (GABA) receptor type A ($GABA_AR$)-mediated evoked inhibitory postsynaptic currents (eIPSCs), recorded from VTA DA neurons, under conditions of potassium channels blockade. The potentiation of these eIPSCs lasted for more than twenty minutes, could be mimicked by activation of D2-like but not D1-like DA receptors, and was accompanied by an increase in the frequency of $GABA_AR$-mediated spontaneous miniature inhibitory postsynaptic currents (mIPSCs). Furthermore, exposure to inhibitors of DA transporter (DAT) led to potentiation of $GABA_A$ currents in a manner similar to the DA-mediated potentiation. Finally, a prolonged presence of L-NAME, an inhibitor of nitric-oxide (NO) signaling was found to conceal the potentiation of $GABA_A$ currents induced by the DA-related drugs. Taken together, this study demonstrates a new modulatory form of VTA $GABA_A$ neurotransmission mediated by DA-related drugs. These results also suggest better understanding of the initial inhibitory action of DA-related drugs on the activity of DA neurons in the VTA.

1. Introduction

Following exposure to various drugs, the extracellular concentration of dopamine (DA) in the ventral tegmental area (VTA) increases (Bradberry and Roth, 1989; Dichiara and Imperato, 1988), thus more interaction with corresponding DA receptors is obtainable. DA receptors are G-protein coupled-receptors (GPCR), divided into the D1-like class, including D1 and D5 receptors (D1-like receptors), and the D2-like class, including D2, D3, and D4 receptors (D2-like receptors) (Missale et al., 1998). Accumulative evidence indicates that a transient elevation of DA concentration in the VTA causes a variety of synaptic modifications, which eventually affect the overall activity of VTA DA neurons. Both excitatory and inhibitory inputs to VTA DA neurons were shown to be modulated by DA-related drugs. These modulations either appear immediately following drug exposure or in a delayed manner, and can be transient or prolonged (Liu et al., 2005; Michaeli and Yaka, 2010; Pan et al., 2008; Schilstrom et al., 2006; Ungless et al., 2001). An early change in the neuronal activity of DA neurons has been demonstrated using *in vivo* electrophysiological recordings from rat VTA. Specifically, exposure to either cocaine or other inhibitors of DA transporter (DAT) profoundly inhibits the activity of VTA DA neurons. The exact mechanism underlying this reduction in neuronal activity is not yet clear. However, it has been suggested that an emergence of negative feedback from γ-amino-butyric acid (GABA) afferents is involved (Bunney and Aghajanian, 1976, 1978; Bunney et al., 1973; Einhorn et al., 1988). We recently reported that DA induces a transient inhibition of GABA receptor type A ($GABA_AR$)-mediated evoked inhibitory postsynaptic currents (eIPSCs) in VTA DA neurons *in vitro*. This inhibition was shown to result from activation of D2-like receptors and enhancement of the conductance of presynaptic potassium currents (Michaeli and Yaka, 2010). In the current study, a DA-induced prolonged potentiation,

Abbreviations: $BaCl_2$, barium-chloride; DA, dopamine; DAT, dopamine transporter; eIPSC, evoked-IPSC; $GABA_AR$, GABA receptor type A; mIPSC, miniature IPSC; NO, nitric-oxide; NOS, nitric-oxide synthase; TTX, tetrodotoxin; VSSC, voltage-sensitive sodium-channels; VTA, ventral tegmental area.

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rather than transient inhibition, of $GABA_A$ currents in VTA DA neurons is presented. This potentiation could only be noticed under blockade of potassium channels, which previously were shown to underlie the transient inhibition of $GABA_A$ eIPSCs caused by DA. The potentiation of $GABA_A$ currents was found to be mediated by D2-like receptors, and could be mimicked by exposure to DAT inhibitors as well. These findings suggest a new form of modulation of inhibitory synapses in the VTA by DA-related drugs, and contribute to the understanding of the mechanism underlying the initial inhibition of VTA DA neuron activity following exposure to DAT inhibitors.

2. Materials 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 18–24-day-old male Sabra rats, a strain of the Hebrew University. An effort was made to minimize animal suffering and to reduce the number of animals used.

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 aCSF 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 one hour before being placed in a recording chamber with a continuous flow (2 ml/min) of aCSF solution (32–34 °C). All aCSF 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): 144 KCl, 1 $CaCl_2$, 3.45 K_4BAPTA, 10 HEPES, 2 Mg_2ATP, and 0.25 NaGTP, pH 7.2–7.4. All the recordings were performed at a holding potential of −70 mV. Series and input resistance were monitored continuously with a 4 mV depolarizing step delivered before every electrical stimulus and experiments were begun only after series resistance had stabilized. Data were discarded if series resistance changed by more than 15%. Due to the composition of the internal solution, $GABA_A$R-mediated currents were inward at this membrane potential, and were blocked completely by the $GABA_A$R antagonist picrotoxin (100 μM; data not shown). 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. All $GABA_A$R-mediated currents were recorded in the constant presence of AP5 (D-(−)-2-Amino-5-phosphonopentanoic acid; 50 μM), CNQX (6-Cyano-7-nitroquinoxaline-2,3-dione; 10 μM) and strychnine (1 μM) to block N-methyl-D-aspartate (NMDA) receptor-, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor- and glycine receptor-mediated currents, respectively. For eIPSC recordings, 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 0.05 or 0.1 Hz. DA solution (dissolved in aCSF) was prepared immediately before its addition to the bath solution. Unless otherwise mentioned, all drugs were added to the bath solution for 10 min. Spontaneous miniature inhibitory postsynaptic currents (mIPSCs) were collected (2 min) in the presence of tetrodotoxin (TTX; 1 μM) to block action potentials. Slices used for recording were discarded after each experimental procedure. Data were filtered at 2 kHz, digitized at 10 kHz, and collected on-line. For eIPSC recordings, Igor Pro acquisition software (Lake Oswego, OR) was used. Ih-currents and mIPSC recordings were performed using a Digidata 1440A digitizer and an acquisition software pClamp 10 (Molecular Devices, Foster City, CA). All chemicals, including quinpirole (trans-(−)-(4aR)-4,4a,5,6,7,8,8a, 9-Octahydro-5-propyl-1H-pyrazolo[3,4-g]quinoline monohydrochloride), SKF81297 (R-(+)-6-Chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydro bromide), eticlopride (S-(−)-3-Chloro-5-ethyl-N-[(1-ethyl-2-pyrrolidinyl)methyl]-6-hydroxy-2-methoxybenzamide hydrochloride), GBR12909 (1-(2-[bis(4-Fluorophenyl) methoxy]ethyl)-4-(3-phenylpropyl)piperazine dihydrochloride), and L-NAME (N^G-Nitro-L-arginine methyl ester), were purchased from Sigma–Aldrich (St. Louis, MO), except APV, CNQX, and PTIO (2-(4-Carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide), which were purchased from Tocris Bioscience (Bristol, UK) and TTX and rTertiapin-Q from Alomone (Jerusalem, Israel).

2.4. Data analysis

Electrophysiological results were normalized to baseline or control state as mentioned. The amplitude of eIPSCs was calculated by measuring the difference between the peak of the current and the baseline current immediately before the stimulus artifact. Unless otherwise specified, the changes in eIPSC amplitude shown by time-course graphs were calculated by comparing the average amplitude of eIPSCs 15–20 min after the end of the treatment with that of successive 5 min before the treatment. Sample traces shown in the figures represent an average of 12 traces. To accurately determine the amplitude and frequency of mIPSCs, only mIPSCs $\geq$ 10 pA were included in the analysis, which was performed using the MiniAnalysis software (Synaptosoft, Decatur, GA). Data sets were compared using *t*-test, paired *t*-test, or one way ANOVA, followed by Tukey's *post hoc* analysis, and Kolmogorov–Smirnov (KS) test at a significance level of 0.05. All values are presented as average ± SEM.

3. Results

3.1. The presence of potassium channel blockers exposes a DA-induced delayed increase in the amplitude of $GABA_A$R eIPSCs, in VTA DA neurons

Drugs associated with acute elevation of DA concentration in the VTA are known to cause postponed prolonged synaptic modulations in the VTA (Bellone and Luscher, 2006; Faleiro et al., 2004; Liu et al., 2005; Ungless et al., 2001). As previously reported (Michaeli and Yaka, 2010), under normal recording conditions, DA transiently reduced $GABA_A$R eIPSCs. Furthermore, no additional effect was observed during the 20 min subsequent to either 10 μM or 30 μM DA exposures (104 ± 8% of baseline; $n = 5$; $p > 0.05$ and 95 ± 8% of baseline; $n = 5$; $p > 0.05$, respectively) (Fig. 1). However, in the presence of 2 mM barium-chloride ($BaCl_2$), a nonspecific potassium channel blocker that was found both previously (with 30 μM DA) and in the present study to prevent the DA-induced $GABA_A$R eIPSC inhibition (10 μM DA, 104 ± 7% of baseline; $n = 13$; $p > 0.05$; 30 μM DA, 103 ± 6% of baseline; $n = 8$; $p > 0.05$; 3 μM DA, 109 ± 8% of baseline; $n = 5$; $p > 0.05$; last 5 min of DA exposure) (Fig. 2*e*), a delayed increase in the amplitude of $GABA_A$R eIPSCs was observed. Compared to the transient and immediate inhibition of $GABA_A$ eIPSCs caused by DA, the current effect began to gradually develop several minutes after DA exposure and persisted for at least 20 min. This increase did not linearly depend on the concentration of DA, since the maximal effect was obtained with 10 μM DA (133 ± 9% of baseline; $n = 7$; $p < 0.001$), compared to 30 and 3 μM DA (122 ± 12% of baseline; $n = 5$; $p < 0.005$ and 115 ± 7% of baseline; $n = 5$; $p < 0.05$, respectively) (Fig. 2). It is important to note that $BaCl_2$ itself produces an increase in $GABA_A$R eIPSCs (data not shown) as previously described (Kombian et al., 2003; Mouginot et al., 1998), however, our recordings were conducted when this increase was stabilized. A similar DA-induced delayed enhancement of $GABA_A$R eIPSCs was observed in the presence of 200 μM rTertiapin-Q, a specific inhibitor of G-protein-coupled inwardly-rectifying potassium current (GIRK) channels (data not shown). Together, these findings describe a novel form of modulation of $GABA_A$R eIPSCs *in vitro*, induced by DA exposure.

3.2. The DA-induced increase in $GABA_A$R eIPSCs is mediated by D2-like receptor

Theoretically, both D1-like and D2-like receptors might mediate the DA-induced potentiation of $GABA_A$R eIPSCs. Therefore, a series of experiments, all in the presence of $BaCl_2$, was conducted in order to study the potential involvement of D1-like and D2-like receptors in this synaptic modulation. We found that quinpirole (D2-like receptor agonist; 10 μM) potentiated $GABA_A$R eIPSCs in a manner