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Field potential recording in the ventral tegmental area: Pharmacological and toxicological evaluations of postsynaptic dopaminergic neuron activity

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Abstract

Addictive drugs and psychologic stress influence the input strength of ventral tegmental area (VTA) neurons, which implies the involvement of synaptic plasticity in dopaminergic neurons. Properties of excitatory synaptic transmission to the dopaminergic neurons have been analyzed using intracellular and patch-clamp recording methods. In the present study, we attempted to establish the field recording procedure in VTA slice preparations to monitor excitatory synaptic transmission. We evaluated this procedure using slice preparations from 6-hydroxydopamine (6-OHDA)-treated animals. In horizontal slices containing the VTA, electrical stimulation of anterior afferent fibers produced two distinct negative field potentials, presumably a fiber volley component and a transsynaptic component. Pharmacological analysis revealed that the transsynaptic component was composed of bicuculline-sensitive and CNQX-sensitive components. Neonatal 6-OHDA administration reduced approximately 90% of tyrosine hydroxylase expression in the VTA and eliminated more than 50% of the transsynaptic components. This result suggests that at least 50% of the observed transsynaptic component reflected the postsynaptic responses of the dopaminergic neurons.

Keywords: VTA; Field potential; Excitatory transmission; 6-Hydroxydopamine

1. Introduction

Anatomically, dopaminergic neurons in the ventral tegmental area (VTA) receive glutamatergic inputs mainly from the medial frontal cortex (Sesack and Pickel, 1992; Carr and Sesack, 2000b), and GABAergic inputs from the nucleus accumbens and ventral pallidum (Geisler and Zahm, 2005). The VTA contains two major neuronal populations: dopaminergic neurons (50–80%) and GABAergic neurons (20–50%), which regulate the excitation of the dopaminergic neurons (Swanson, 1982; Henry and White, 1992; Johnson and North, 1992; Van Bockstaele and Pickel, 1995; Steffensen et al., 1998; Carr and Sesack, 2000a). Patch-clamp techniques have been applied to characterize the pharmacological properties and plasticity of excitatory input to these types of VTA neurons (Bonci and Malenka, 1999; Jones and Kauer, 1999; Jones et al., 2000; Koga and Momiyama, 2000).

Addictive drugs and stress enhance excitatory synaptic responses of VTA neurons (Ungless et al., 2001; Saal et al., 2003; Dong et al., 2004; Faleiro et al., 2004). Synaptic plasticity of dopaminergic neurons, in particular, has a crucial role in establishing drug addiction, such as drug-associated conditioned behaviors (Harris and Aston-Jones, 2003; Dong et al., 2004). Therefore, a convenient method to monitor synaptic potentials using field potential recordings is useful for the analysis of synaptic input activities and synaptic plasticity in dopaminergic neurons. The properties of evoked synaptic potentials in dopaminergic neurons have been analyzed pharmacologically using horizontal VTA slice preparations (Johnson and North, 1992; Bonci and Malenka, 1999). Field potentials in the VTA, however, have not been analyzed in the horizontal slice preparation.

A dopaminergic toxin, 6-hydroxydopamine (6-OHDA), is preferentially taken up by catecholaminergic neurons via catecholamine transporters. 6-OHDA directly inhibits mitochondrial respiratory chain complexes or produces oxidative stress via hydrogen peroxide to induce neural degeneration

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(Glinka et al., 1997; Blum et al., 2001). Midbrain dopaminergic neurons degenerate both in the substantia nigra pars compacta and VTA, depending on the amount and site of the 6-OHDA injection (Blum et al., 2001; Muma et al., 2001; Norton et al., 2002; Rodriguez et al., 2001; Brot et al., 2002). In the present study, we attempted to establish field recording procedures for VTA neurons in horizontal slice preparations. In particular, dopaminergic lesion model was used to evaluate the contribution of synaptic response of dopaminergic neurons to VTA field potentials.

2. Methods

2.1. 6-Hydroxydopamine (6-OHDA) injection

We performed intracranial injection of 6-OHDA in postnatal day (P) 3–9 Sprague-Dawley rats (Brot et al., 2002). Juvenile rats (P16–19) with 6-OHDA lesions were used for electrophysiological recording. The pups were injected intraperitoneally with 10 μl of 25 mg/kg desipramine (Sigma Chemical Co., St. Louis, MO), a norepinephrine uptake blocker. After 60 min, the pups were then anesthetized by inhalation of halothane (Takeda Chemical Industries Ltd., Osaka, Japan), and intracranially injected with 6-OHDA (10 μg/μl, 10 μl in saline with 0.05% ascorbic acid; Sigma), using a 10 μl Hamilton syringe connected to a dental needle (30 gauge, TERUMO, Tokyo, Japan). The same volume of saline with 0.05% ascorbic acid was injected in control animals. The needle position was 4–5 mm anterior from lambda and 2 mm lateral from midline at a depth of 4–5 mm. In case of single intra-ventricular injection, VTA dopaminergic neurons are less vulnerable to 6-OHDA (Rodriguez et al., 2001; Brot et al., 2002). Therefore, we injected 6-OHDA one to four times every other day starting at P3. Administrative condition for electrophysiological experiments was determined by the level of tyrosine hydroxylase (TH) and the number of TH-immunoreactive cells in the VTA at P16 (see below, Fig. 4).

2.2. Slice preparation

Rats (P16–19) were anesthetized with halothane, and then decapitated. Brains were removed into cold high sucrose artificial cerebrospinal fluid (ACSF) containing the following reagent (in mM): 195 sucrose, 1 NaH_2PO_4, 2.5 KCl, 5 $MgSO_4$, 1.0 $CaCl_2$, 26.2 $NaHCO_3$, 11 D-glucose, 1 ascorbic acid, pH 7.4, and saturated with 95% O_2 and 5% CO_2. Horizontal slices containing the VTA were prepared according to Johnson and North (1992). Briefly, a block containing the midbrain was placed ventral side-up in a microslicer (DTK-2000, Dosaka, Kyoto, Japan). Slices were discarded until the floor of the interpeduncular fossa was reached; the next slice (thickness: 500 μm) was dissected at the midline. These slices were placed in an incubation chamber for at least 1 h at room temperature. The chamber was filled with ACSF containing the following (in mM): 119 NaCl, 1.0 NaH_2PO_4, 2.5 KCl, 1.3 $MgSO_4$, 2.5 $CaCl_2$, 26.2 $NaHCO_3$, 11 D-glucose, and 1 ascorbic acid.

2.3. Electrophysiology

Electrophysiological experiments were performed at room temperature (24–26 °C). Slices were placed in the recording chamber, which was continuously perfused with normal ascorbic acid-free ACSF at ∼2.0 ml/min. The VTA was identified as the region lateral to the fasciculus retroflexus (fr) and medial to the medial terminal nucleus of the accessory optic tract (MT) (Johnson and North, 1992; Paxinos and Watson, 1998). A glass microelectrode filled with ACSF (5–8 MΩ) was placed in the middle position between the fr and MT (Fig. 1). The depth of the electrode was adjusted to obtain a maximal response. Field potentials were evoked with a micro-concentric bipolar stimulating electrode (tip-diameter: 25 μm; MCE-100, David Kopf Instruments, Tujunga, CA). The stimulating electrode was typically placed ∼350 μm antero-laterally from the recording electrode at an angle of 30–45° from the midline (see Fig. 2A). With this electrode configuration, a 0.6 mA electrical stimulation produced a field response with a first negative potential (N1: see Fig. 3) of 0.7–1.8 mV. Repetitive stimulus pulses (duration: 50 μs, stimulus intensity: 0.2–1.0 mA) were applied at 0.05 Hz to evoke field potentials. The evoked potentials were amplified by a microelectrode amplifier (MEZ-8301, Nihonkoden. Japan) via a high gain amplifier (AVH-11, Nihonkoden. Japan). The amplified signal was digitized at 2 kHz (DigiData 1200, Axon Instruments, Sunnyvale, CA, USA). Data were acquired using Clampex 7 (Axon Instruments). For analyses of the amplitude of field potentials (see Fig. 5), five traces were averaged for each stimulus intensity, using Clampfit 6 (Axon Instruments).

For voltage-clamp analysis, individual cells between the fr and MT were visualized with an upright microscope, and whole-cell recordings were made by using an Axopatch 200B, according to Nagano et al. (2003). Dopaminergic neurons were physiologically identified by their hyperpolarization-induced 'sag' potential (Johnson and North, 1992).

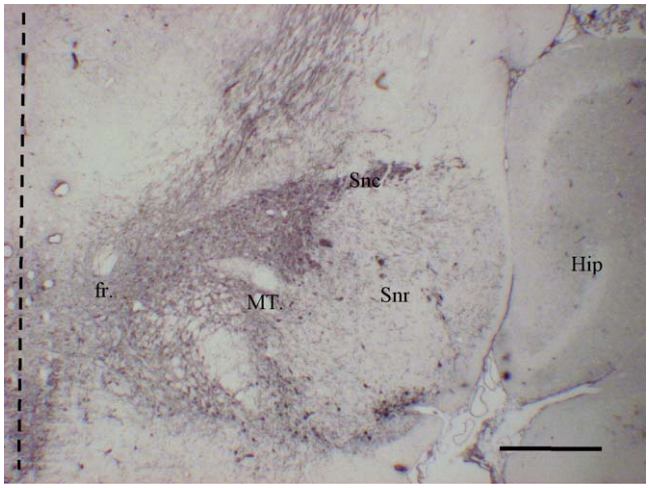

Fig. 1. Identification of ventral tegmental area (VTA) in the horizontal section including midbrain. The VTA was identified according to Paxinos and Watson (1998). The section was stained with anti-tyrosine hydroxylase (TH) antibody and visualized using the ABC-diaminobenzidine mothod with nickel intensification. fr: fasciculus retroflexus, MT: medial terminal nucleus of the accessory optic tract, Snc: substantia nigra compacta, Snr: substantia nigra reticulata, Hip: hippocampus. The dashed line indicates midline. Scale bar: 500 μm.

2.4. Western blotting

A coronal slice (1 mm of thick) including the VTA was prepared in cold high sucrose ACSF. The VTA region between both sides of MT (Paxinos and Watson, 1998) was dissected and homogenized with Laemmli sample buffer (2% sodium dodecyl sulfate (SDS), 62.5 mM Tris (pH 6.8)). After centrifugation, the protein in the supernatants was denatured at 95 °C in the presence of 5% 2-mercaptoethanol and 10% glycerol. Protein (20 μg/lane) was separated by SDS-polyacrylamide gel electrophoresis and transferred to a polyvinylidine difluoride membrane by electrophoresis. Primary antibodies (monoclonal anti-TH antibody, 1:1000, Iwakura et al., 2005; monoclonal anti-β-actin antibody, 1:1000, Chemicon International, Temecula, CA; polyclonal anti-GAD antibody, 1:3000, Sigma) were diluted and incubated with the membrane at 4 °C overnight. Immunoreactivity was detected with goat anti-mouse or rabbit immunoglobulin conjugated to peroxidase (1:10000; Vector Laboratories, Burlingame, CA), followed by chemiluminescence reaction (ECL kit, Amersham Biosciences, Tokyo, Japan).

2.5. Immunohistochemistry

Rats were anesthetized with halothane and sacrificed by transcardial perfusion with 4% paraformaldehyde in 0.1 M phosphate-buffered solution (pH 7.4). The brains were removed and postfixed in the same solution for 8–12 h. The tissues were sectioned at 12 μm using a cryostat (CM1510, Leica, Nussloch, Germany). The sections were incubated with monoclonal anti-TH antibody. The immunoreacitivity was detected with horse anti-mouse immu-