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DOPAMINE INHIBITS GABA TRANSMISSION FROM THE GLOBUS PALLIDUS TO THE THALAMIC RETICULAR NUCLEUS VIA PRESYNAPTIC D4 RECEPTORS

D. GASCA-MARTINEZ,[a] A. HERNANDEZ,[b] A. SIERRA,[c] R. VALDIOSERA,[c] V. ANAYA-MARTINEZ,[d] B. FLORAN,[c] D. ERLIJ[c] AND J. ACEVES[c]*

[a]*Departamento de Farmacología, CINVESTAV-IPN, Apartado postal 14-740, México D.F., 07000, México*

[b]*Division de Neurociencias, Instituto de Fisiología Celular UNAM, Apartado postal 70-253, México D.F., 04510, México*

[c]*Departamento de Fisiología, Biofísica y Neurociencias, CINVESTAV-IPN, Apartado postal 14-740, México D.F., 07000, México*

[d]*Departamento de Neurociencias, UNAM-Iztacala, Apartado postal 314, Los Reyes Iztacala, Edo. de México, 54090 México*

Abstract—The globus pallidus sends a significant GABAergic projection to the thalamic reticular nucleus. Because pallidal neurons express D4-dopamine receptors, we have explored their presence on pallidoreticular terminals by studying the effect of dopamine and D4-receptor agonists on the GABAergic transmission in the thalamic reticular nucleus. We made whole-cell recordings of inhibitory postsynaptic currents (IPSCs) and miniature inhibitory postsynaptic currents (mIPSCs) in the thalamic reticular neurons. Dopamine consistently reduced the IPSCs. The effect of dopamine was associated with paired-pulse facilitation, indicating a presynaptic location of the receptors. The effect of dopamine was also measured on the mIPSCs, reducing their frequency but not affecting their amplitude, which also suggests a presynaptic site of action. The selective D4-receptor agonist PD 168,077 also reduced the IPSCs, which was also associated with paired-pulse facilitation. In addition, this agonist reduced the frequency of the mIPSCs with no effect on their amplitude. The D4-receptor antagonist L-745,870 totally blocked the effect of the D4-receptor agonist, indicating the specificity of its effect. To verify the location of the receptors on the pallidal terminals, these were eliminated by injecting kainic acid into the globus pallidus. Kainic acid produced a drastic (80%) fall in the globus pallidus neuronal population. In this condition, the effect of the activation of D4 receptors both on the IPSCs and mIPSCs was prevented, thus indicating that the location of the receptors was on the pallidal terminals. Our results demonstrate that dopamine controls the activity of the thalamic reticular neurons by regulating the inhibitory input from the globus pallidus.

Key words: basal ganglia, dopamine receptors, D4-agonists, Parkinson's disease, sleep disorders, thalamus.

The presence of markers of dopamine transmission in the thalamus has been known for some time in rodents, primates, and humans (Wang et al., 1995; Freeman et al., 2001; Sanchez-Gonzalez et al., 2005). In the rat, the dopaminergic innervation of the thalamus originates from the pars compacta of the substantia nigra (Freeman et al., 2001; Prensa and Parent, 2001; Anaya-Martinez et al., 2006). The innervation is particularly significant in the thalamic reticular nucleus (TRn), as shown by the high density of the dopamine transporter and the immunolabeling for D1- and D4-dopamine receptors (Huang et al., 1992; Mrzljak et al., 1996; Khan et al., 1998; Freeman et al., 2001). The TRn is made of a group of GABAergic neurons (Houser et al., 1980; De Biasi et al., 1986) that modulate the flow of information through the thalamus and that is central for attention, waking, sleep, and the genesis of various types of rhythmic activity (Steriade et al., 1985, 1986; Friedberg and Ross, 1993; von Krosigk et al., 1993; McAlonan et al., 2008).

The TRn receives a significant GABAergic input from the globus pallidus (external segment in primates) in rats (Asanuma, 1989; Cornwall et al., 1990; Gandia et al., 1993), and monkeys (Hazrati and Parent, 1991; Asanuma, 1994). In previous work (Floran et al., 2004a), we showed that the D4-dopamine receptors modulate depolarization-stimulated [^{3}H]GABA release, which indicates the presence of the D4 receptors on GABAergic terminals.

Because neurons of the globus pallidus express D4-dopamine receptors (Mrzljak et al., 1996; Ariano et al., 1997), it is likely that the receptors are transported to the axon terminals in the TRn. We have examined whether the receptors are indeed present on the axon terminals originating in the globus pallidus. We have studied the effect of dopamine and D4-dopamine receptor agonists and antagonists on inhibitory postsynaptic currents (IPSCs) and miniature IPSCs (mIPSCs) on the TRn neurons. The presence of receptors on pallidal terminals was shown by measuring the disappearance of the effects of the activation of the receptors after the lesion of the globus pallidus with kainic acid. Preliminary results of this work have been presented elsewhere (Gasca-Martinez et al., 2009).

*Correspondence to: J. Aceves, Departamento de Fisiología, Biofísica y Neurociencias, CINVESTAV-IPN, Apartado postal 14-740, México D.F., 07000, México. Tel: +52-55-5747-3800 Ext. 5188; fax: +52-55-5747-3754.
E-mail address: jaceves@fisio.cinvestav.mx (J. Aceves).

Abbreviations: ABC, avidin–biotin–peroxidase complex; ACSF, artificial cerebrospinal fluid; CPu, caudate–putamen; DAB, 3,3′-diaminobenzidine tetrahydrochloride; EDS, excessive daytime sleepiness; GP, globus pallidus; ic, internal capsule; IPSCs, inhibitory postsynaptic currents; I_T, low threshold-activation Ca^{2+} current; L-745, L-745,870; mIPSCs, miniature inhibitory postsynaptic currents; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PD, PD 168,077; REM, rapid eye movement; Thal, thalamus; TRn, thalamic reticular nucleus; V_m, membrane potential.

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EXPERIMENTAL PROCEDURES

Slice preparation and solutions

Experimental procedures were done in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the Institutional Animal Care Committees of the CINVESTAV. Brain slices obtained from male Wistar rats (postnatal day 14 to 21) were used. The rats were anesthetized and decapitated. The brain was quickly removed from the skull and placed in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): 124 NaCl, 26 $NaHCO_3$, 2.5 KCl, 1.3 $MgCl_2$, 1.2 NaH_2PO_4, 2.4 $CaCl_2$, and 10 glucose, pH 7.4 (with 95% O_2 and 5% CO_2 bubbling through the solution). To prevent swelling of the cell during slicing, NaCl was replaced with choline chloride. Both solutions were continuously oxygenated with the gas mixture. Horizontal slices (300 μm) containing the TRn (see Fig. 1A) were cut with a vibroslicer (Lancer, Technical Products International, St. Louis, MO, USA) and then transferred to normal ACSF at room temperature (ca 25 °C) for equilibration. After 1 h, a single slice was transferred to a recording chamber continuously superfused with ACSF (1 to 2 mL/min) at room temperature. To block N-methyl-D-aspartate and non-N-methyl-D-aspartate glutamate receptors, AP-5 (50 μM) and CNQX (10 μM) were added to the superfusion medium. TTX (1 μM) was also added when studying the effect of drugs on the mIPSCs.

Electrophysiology

Neurons were visualized using infrared differential-interference video microscopy with a 40× water-immersion objective (Hamamatsu C2400-50, Hamamatsu Photonics Systems, USA and Axioscop, Carl Zeiss, Oberkochen, Germany). Micropipettes for whole cell recordings were pulled (Sutter Instruments, Novato, CA, USA) from borosilicate glass tubes (1.5 mm outer diameter, WPI, Sarasota, FL, USA) for a final resistance of 2 to 5 MΩ when filled with a solution of the composition (in mM): 120 KSO_3CH_3, 16 KCl, 2 $MgCl_2$, 10 HEPES, 1.1 K_2-EGTA, 1.1 ATP-Mg, and 1.1 GTP-Na (pH 7.3 adjusted with KOH; osmolality, 287 to 290 mOsm L^{-1}). This internal solution was used for current-clamp recordings. The IPSCs were recorded in a voltage-clamp condition (holding potential = −80 mV) with pipettes containing a solution of the composition (in mM): 115 CsCl, 5 $MgCl_2$, 10 HEPES, 10 K_2-EGTA, 4 ATP-Mg, and 1 GTP-Na. QX-314 (5 mM) was added to avoid contamination of synaptic responses by unclamped action-currents. The calculated chloride-equilibrium potential was 0 mV. The IPSCs were produced using a bipolar, concentric Pt–Ir electrode (50 μm at the tip, 1 kΩ DC resistance; FHC, Bowdoinham, ME, USA) at a frequency of 0.1 Hz using rectangular pulses (20 μs, 10 to 20 V; Digitimer Ltd isolated stimulator DS2, England). The electrode was placed near (ca 100 μm) the recorded cell. The strength of the pulses was adjusted to produce synaptic currents

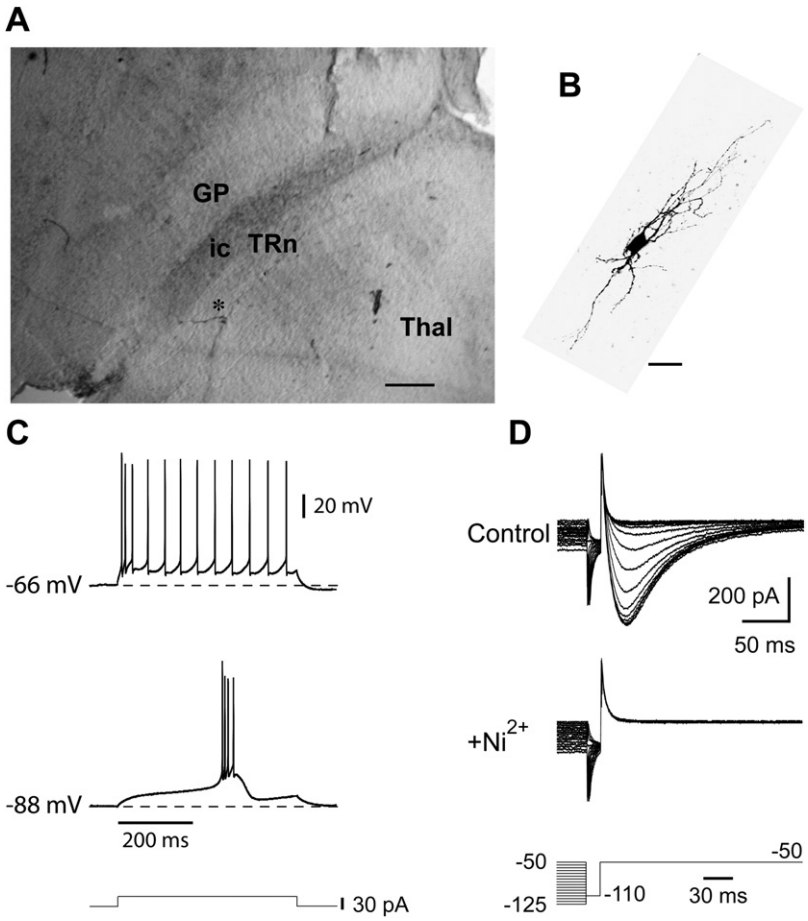

Fig. 1. Firing properties of the recorded neurons. (A) Location of the TRn in the brain slice. The asterisk indicates the location of the neuron shown in (B). (B) Byocitin-filled neuron from which the recording was made. (C) At −66 mV of the membrane potential, a depolarizing current pulse produces tonic firing (upper trace); at −88 mV, the same pulse produces a low-threshold calcium spike that triggers a burst of action potentials. (D) Illustration of the I_T current recorded in another neuron. The lower trace shows that the current was eliminated by Ni^{2+} (1 mM). Scale bars = 400 μm in (A); 27 μm in (B). ic, internal capsule; GP, globus pallidus; Thal, thalamus; TRn, thalamic reticular nucleus.