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Differential voltage-sensitivity of D_2-like dopamine receptors

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ABSTRACT

Agonist potency at some neurotransmitter receptors has been shown to be regulated by transmembrane voltage, a mechanism which has been suggested to play a crucial role in the regulation of neurotransmitter release by autoreceptors and in synaptic plasticity. We have recently described the voltage-sensitivity of the dopamine D_{2L} receptor and we now extend our studies to include the other members of the D_2-like receptor subfamily; the D_{2S}, D_3, and D_4 dopamine receptors. Electrophysiological recordings were performed on *Xenopus* oocytes coexpressing human dopamine D_{2S}, D_3, or D_4 receptors with G protein-coupled potassium (GIRK) channels. Comparison of concentration–response relationships at −80 mV and at 0 mV for dopamine-mediated GIRK activation revealed significant rightward shifts for both D_{2S} and D_4 upon depolarization. In contrast, the concentration–response relationships for D_3-mediated GIRK activation were not appreciably different at the two voltages. Our findings provide new insight into the functional differences of these closely related receptors.

Recently, voltage-sensitivity has been shown for the activation and ligand binding properties of the muscarinic M_1 and M_2, glutamate $mGluR_1$ and $mGluR_3$, and the long isoform of the dopamine D_2 receptor (D_{2L}) [1–3]. In these studies utilizing the *Xenopus* oocyte expression system, significant shifts in the concentration–response relationships for ion channel activation at hyper- and depolarized membrane potentials were observed. Ben-Chaim et al. [1] and Ohana et al. [2] demonstrated that the ligand potency shifts observed in these functional assays correlated with changes in radioligand binding affinity, and Ben-Chaim et al. [4] reported charge movements within the receptor which correlated with the shifts in affinity and potency. The G_q-coupled M_1 and $mGluR_1$ receptors exhibited leftward shifts in agonist potency with depolarization, whereas the $G_{i/o}$-coupled M_2, $mGluR_3$, and D_{2L} exhibited leftward shifts [1–3]. The phenomenon of voltage-sensitivity of G protein-coupled receptors has been proposed to be a novel mechanism of synaptic plasticity and to play an important role in the regulation of neurotransmitter release by presynaptic autoreceptors [1,2].

The D_2-like subfamily of dopamine receptors, the long and short splice isoforms of the dopamine D_2 receptor (D_{2L} and D_{2S}), the dopamine D_3 receptor (D_3), and the dopamine D_4 receptor (D_4), are all coupled to G proteins of the $G_{i/o}$ class, which mediate inhibition of adenylate cyclase and opening of G protein-coupled potassium (GIRK) channels [5,6]. While we have previously demonstrated the voltage-sensitivity of D_{2L} using the GIRK-channel coupling as a readout [3], we here employ this system to characterize the voltage-sensitivity of the other members of the human D_2-like subfamily.

D_{2S} is the predominant presynaptic D_2 receptor isoform and functions as an auto- and heteroreceptor, regulating transmitter release from glutamatergic, GABAergic, and dopaminergic terminals in the striatum [7,8]. D_2 autoreceptors have been postulated to be a major target for the third generation partial agonist antipsychotics, such as aripiprazole [9].

Similarly, the D_3 receptor has also been implicated as a target for the treatment of psychosis and Parkinson's disease, as it is also expressed in the striatum, although it displays a more restricted localization pattern than D_{2L} and D_{2S}, with predominant expression in the nucleus accumbens and the islands of Calleja [10]. D_3 shares the vast majority of its ligands, including anti-Parkinsonian and antipsychotic agents, with the D_2 receptors [5] and has been suggested to act as an autoreceptor, similar to D_{2S}, but this remains a controversial issue [11]. D_3 couples exclusively to $G_{\alpha o}$ proteins, whereas D_{2L} and D_{2S} can activate both $G_{\alpha i}$ and $G_{\alpha o}$ [12].

The D_4 receptor, in contrast, is predominantly expressed on pyramidal cells of the prefrontal cortex and in the amygdala, but also shows some expression in the basal ganglia, both presynaptically on several types of nerve terminals, and postsynaptically on medium spiny neurons [13,14]. The third intracellular loop of human D_4 contains a variable number of tandem repeats (VNTRs) of 16 amino acids, of which there exist three major polymorphisms; $D_{4.2}$, $D_{4.4}$, and $D_{4.7}$. In the present study, we have chosen to study the four-repeat variant ($D_{4.4}$), which represents the most common allele [5].

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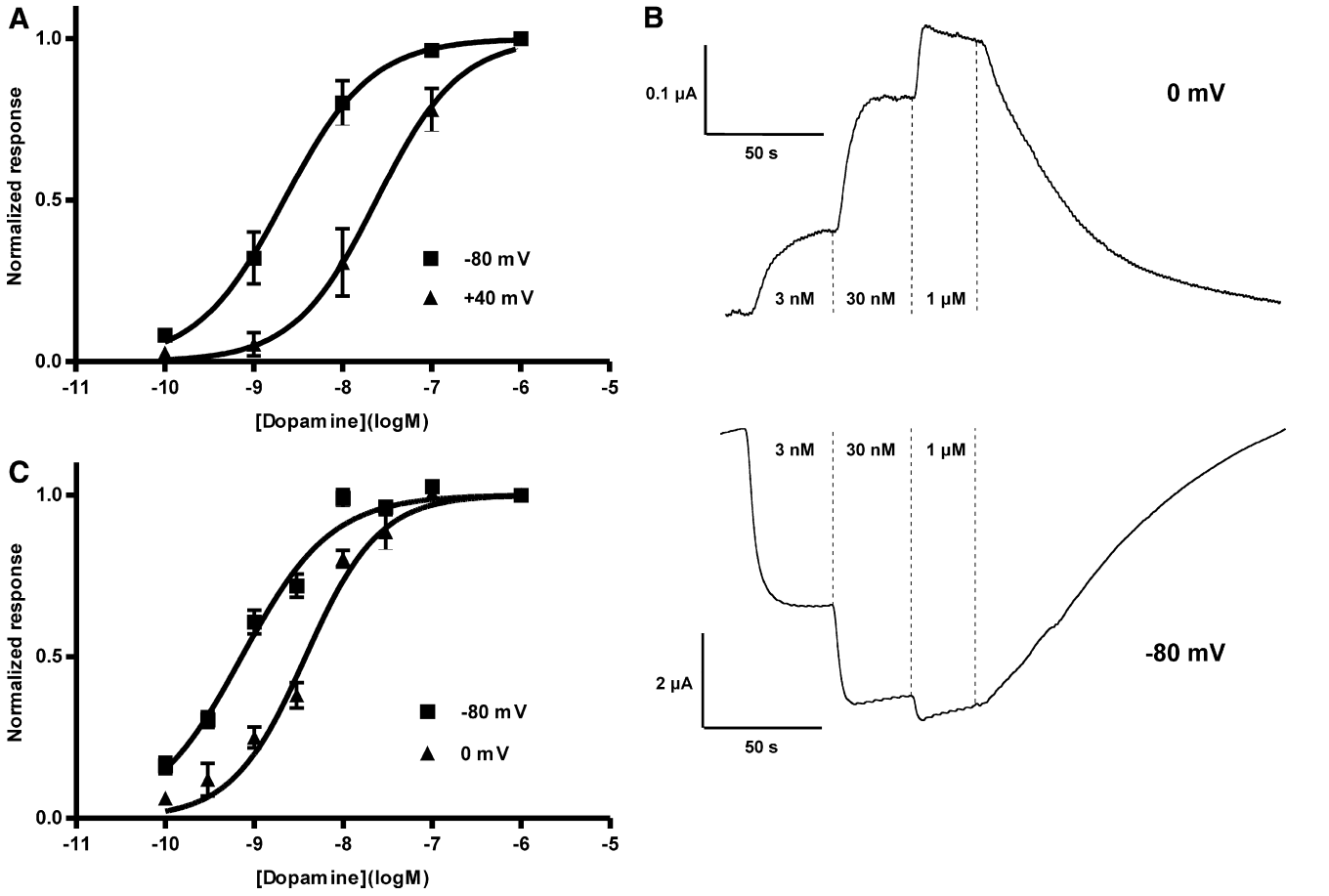

Fig. 1. Voltage-sensitivity of the dopamine D_{2S} receptor. (A) Concentration–response data for dopamine activating GIRK currents via the human dopamine D_{2S} receptor at −80 and +40 mV. The curves were constructed as described in Materials and methods. Each point represents data from three oocytes. The estimated EC_{50}s for dopamine-induced GIRK-channel activation are 2.20 nM [1.55 nM; 3.14 nM] and 24.1 nM [15.9–36.6 nM] at −80 and at +40 mV, respectively (significant difference; $P < 0.001$). (B) Representative current traces showing the GIRK response to increasing concentrations of dopamine at −80 and 0 mV, as indicated, in an oocyte coinjected with cRNA encoding the human dopamine D_{2S} receptor and GIRK1/4 channel subunits. Both traces were recorded in the same oocyte. (C) Concentration–response data for dopamine activating GIRK currents via the human dopamine D_{2S} receptor at −80 and 0 mV. Each point represents data from 3 to 11 oocytes. The estimated EC_{50}s for dopamine-induced GIRK-channel activation are 0.728 nM [0.607 nM; 0.873 nM] and 3.79 nM [3.21 nM; 4.48 nM] at −80 and at 0 mV, respectively (significant difference; $P < 0.001$). The difference between the EC_{50}s at −80 mV in the two experiments in (A) and (C), respectively, derives from the fact that different oocyte batches were used for these recordings, presumably having slightly different receptor expression levels.

We find the D_{2S} receptor to be voltage-sensitive to an extent similar to that of D_{2L}, whereas the dopamine $D_{4.4}$ receptor exhibits weaker modulation by voltage. The potency of D_3 receptor activation by dopamine is, in contrast, insensitive to voltage.

Materials and methods

All studies were performed in accordance with guidelines from the Swedish National Board for Laboratory Animals. Human GIRK1 (Kir3.1) and GIRK4 (Kir3.4) cDNA (provided by Dr. Terence Hebert, University of Montreal, Canada) and cDNA encoding the human dopamine $D_{4.4}$ receptor (purchased from the UMR cDNA resource center, Rolla, MO) were all in pCDNA3.1 (Invitrogen). cDNA encoding the human dopamine D_3 receptor (in pRC-CMV; Invitrogen) was a gift from Dr. Jean-Charles Schwartz (Centre Paul Broca de l'Inserm, Paris, France) and the human dopamine D_{2S} receptor (in pGEM4Z; Promega) was obtained from the laboratory of Dr. Marc Caron (Duke University Medical Center, Durham, NC). cDNA encoding the human $D_{3/2}$ chimera receptor (in pCDNA3.1; see Results and discussion, below, for construct details) was obtained from the laboratory of Dr. Graeme Milligan (The University of Glasgow, Glasgow, UK). The plasmids were linearized with NotI (GIRK1 and GIRK4), SalI (D_{2S} and D_3), and XbaI ($D_{3/2}$). Oocytes were harvested from *Xenopus laevis* toads and injected with cRNA transcribed *in vitro* from the linearized plasmids as described [15]. One nanogram/oocyte of each GIRK subunit cRNA was injected. For the D_{2S}, $D_{4.4}$, D_3, and $D_{3/2}$ receptors, 5, 28, 75, and 56 ng of cRNA, respectively, was injected per oocyte. The volume injected (50 nl/oocyte) was kept constant. The amounts of receptor cRNA injected were chosen to give optimal response amplitudes, and are higher for the D_3 and the $D_{3/2}$, since the D_3 receptor expresses poorly in oocytes [6].

The electrophysiological experiments were performed using a two-electrode voltage-clamp setup as described [3], 4–7 days after cRNA injection. Experiments were carried out at room temperature (20–22 °C). For recording of GIRK currents, a high-potassium solution (64 mM NaCl, 25 mM KCl, 0.8 mM $MgCl_2$, 0.4 mM $CaCl_2$, 15 mM Hepes, and 1 mM acetic acid, adjusted to pH 7.4) was used, giving a K^+ reversal potential of about −40 mV. Two types of pulse protocols were used to evoke GIRK currents from a holding potential of −40 mV; a 6-s ramp from −80 to +40 mV was used to visualize the voltage-dependence of channel opening, whereas single −80, 0, or +40 mV pulses of longer duration were applied to study current responses to dopamine receptor ligand application.

Dopamine (Sigma–Aldrich) was diluted to the desired concentrations in the recording solution before experiments and added