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Research Report

The neuroleptic drug, fluphenazine, blocks neuronal voltage-gated sodium channels

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ABSTRACT

Fluphenazine (Prolixin®) is a potent phenothiazine-based dopamine receptor antagonist, first introduced into clinical practice in the late 1950s as a novel antipsychotic. The drug emerged as a 'hit' during a routine ion channel screening assay, the present studies describe our electrophysiological examination of fluphenazine at tetrodotoxin-sensitive (TTX-S) and resistant (TTX-R) voltage-gated sodium channel variants expressed in three different cell populations. Constitutively expressed TTX-S conductances were studied in ND7/23 cells (a dorsal root ganglion-derived clonal cell line) and rat primary cerebrocortical neurons. Recombinant rat $Na_V1.8$ currents were studied using ND7/23 cells as a host line for heterologous expression. Sodium currents were examined using standard whole-cell voltage-clamp electrophysiology. Current–voltage relationships for either ND7/23 cell or $Na_V1.8$ currents revealed a prominent fluphenazine block of sodium channel activity. Steady-state inactivation curves were shifted by ~10 mV in the hyperpolarizing direction by fluphenazine (3 μM for ND7/23 currents and 10 μM for $Na_V1.8$), suggesting that the drug stabilizes the inactivated channel state. Fluphenazine's apparent potency for blocking either ND7/23 or $Na_V1.8$ sodium channels was increased by membrane depolarization, corresponding IC_{50} values for the ND7/23 cell conductances were 18 μM and 960 nM at holding potentials of –120 mV and –50 mV, respectively. Frequency-dependent channel block was evident for each of the cell/channel variants, again suggesting a preferential binding to inactivated channel state(s). These experiments show fluphenazine to be capable of blocking neuronal sodium channels. Several unusual pharmacokinetic features of this drug suggest that sodium channel block may contribute to the overall clinical profile of this classical neuroleptic agent.

1. Introduction

Fluphenazine (Prolixin®; Fig. 1) is a potent phenothiazine antipsychotic that was introduced into clinical practice in the late 1950s (Darling, 1959; Taylor, 1959). At the time, the drug was considered a significant improvement on existing phenothiazine-based medications because it had no tendency to induce akathisia and a much reduced propensity to flatten affect (Darling, 1959). In addition to its neuroleptic action, the drug has also found occasional use as an anti-emetic (Bellville et al., 1960) and as an adjunct medication to alleviate the pain associated with post-herpetic neuralgia (Graff-Radford et al., 2000; Hurtig, 1990). Fluphenazine is a high affinity ligand for both D_1 and D_2 dopamine receptor

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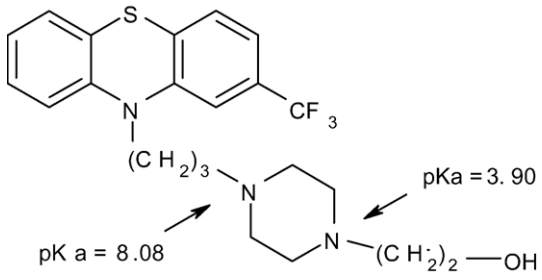

Fig. 1 – Fluphenazine, relative pK_a values for two basic nitrogens are shown.

subtypes (K_i: D_1 = 3.2 nM, D_2 = 0.7 nM; Morgan and Finch, 1986). Potent dopamine receptor antagonism provides a mechanistic basis for the antipsychotic effects of the drug but it also confers many of the major side effects, one of the most prominent being the tendency to promote extrapyramidal dyskinesias (Darling, 1959; Levinson et al., 1990). Though it is not uncommon for fluphenazine and other classical D_2-receptor antagonists to be prescribed for maintenance therapy for psychosis, the overall adverse side effect profile associated with the drug has curtailed its use in the clinic in favour of safer and generally more effective medications (for example, see Dossenbach et al., 2004).

A clinically-effective neuroleptic profile is represented by strikingly diverse structural chemotypes. Many of these drugs, particularly the older generation phenothiazine derivatives, show a broad-spectrum pharmacological profile. For the most part, pharmacological promiscuity appeared to be principally due to an affinity for several G-protein-coupled receptor families. However, more recent studies have begun to reveal an action of several neuroleptic drugs at multiple ion channels. Pimozide and penfluridol (diphenylbutylpiperidines), haloperidol (a butyrophenone) and flunarizine (a diphenyldiperazine), for example, have recently been described as effective blockers of high-voltage-activated (L-type; Ito et al., 1996) or low-voltage-activated (T-type; Santi et al., 2002) calcium channel subtypes. Likewise, similar activity has been reported for several antispychotic drugs, including the phenothiazines, chlorpromazine and fluphenazine, at isoforms of calcium-activated potassium channels (Lee et al., 1997; Terstappen et al., 2001). Chlorpromazine has also been reported to block cardiac sodium channels (Ogata and Narahashi, 1989) and neuronal sodium and calcium channels (Ogata et al., 1990). Finally, off-target effects at the hERG potassium channel in particular, have unearthed a significant cardiac liability of several antipyschotic drugs (Kongsamut et al., 2002), including chlorpromazine (Lee et al., 2004; Thomas et al., 2003) though there have been no such studies yet reported for fluphenazine. Thus, it would appear that ion channels in general represent additional targets for this drug class and, as such, further undermine pharmacological specificity.

Fluphenazine first came to our attention as an extremely potent sodium channel blocker during the course of a routine validation exercise for a high-throughput fluorescence-based assay, designed to detect novel compounds with sodium channel blocking properties. The drug was included along with other commercially available compounds, in a multi-plate library of pharmacologically-active compounds assembled to cover a diverse range of receptor targets. Fluphenazine repeatedly emerged as a potent sodium channel blocker during the course of these experiments. The reproducibility and magnitude of the block seen in the screening assay prompted us to further explore the properties of fluphenazine using native (ND7/23 cell line—a DRG-neuroblastoma hybrid and rat cerebrocortical neurons) and cloned (rat $Na_V1.8$) sodium channel variants under more rigorous voltage-clamp conditions. We were interested to discover whether sodium channel block by fluphenazine might contribute, in some way, to the effects associated with this medication. The data generated from the electrophysiological experiments revealed fluphenazine to be capable of exerting a prominent sodium channel blockade. Certain features of this channel block shared characteristics that are common to a number of other pharmacologically active compounds that act at the 'local anaesthetic' pharmacophore within the sodium channel α subunit. Though the affinity of fluphenazine for the sodium channel binding site is considerably lower than that for D_2 receptors, the high lipophilicity of the drug and its known accumulation in lipid-rich tissues suggest that sodium channel block may contribute to the overall pharmacological profile of this drug.

2. Results

2.1. *Basic features of the block of voltage-gated sodium channels by fluphenazine*

We have reported previously on the characteristics of voltage-gated sodium channel conductances in ND7/23 cells (Zhou et al., 2003). A prominent, transient, TTX-S inward sodium current can be evoked in this neuroblastoma cell line by brief depolarizations from negative holding potentials. Preliminary experiments revealed that negative membrane potentials in the range of –120 mV ensured negligible steady-state inactivation and, hence, maximal sodium channel availability. Sequential step depolarizations from –120 mV to command voltages more positive than –40 mV elicited typical, fast inactivating, inward sodium currents (Fig. 2A) that peaked at around –10 mV and reversed direction at around +70 mV, i.e., close to the predicted sodium equilibrium potential (Fig. 2B). Bath application of fluphenazine (10 μM) reversibly attenuated the inward sodium currents by approximately 50% at all test voltages. When the data were adjusted for the change in driving force at each membrane potential, the resulting conductance–voltage plot revealed that fluphenazine's action was not the result of an effect on sodium channel activation characteristics (Fig. 2C).

The ND7/23 cell line is also a suitable host cell line for the expression of recombinant rat $Na_V1.8$ sodium channels. The TTX-R current attributed to the latter could be studied in isolation from the background TTX-S conductance in the wild-type cells by the inclusion of TTX (10 μM) in the bathing