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Flunarizine blocks voltage-gated Na^+ and Ca^{2+} currents in cultured rat cortical neurons: A possible locus of action in the prevention of migraine

Qing Ye[a], Lan-Yun Yan[a], Liu-Jun Xue[a], Qiang Wang[b], Zhi-Kui Zhou[a], Hiang Xiao[b], Qi Wan[a,*]

[a] Department of Neurology, the First Affiliated Hospital of Nanjing Medical University, Guangzhou Street 300, Nanjing, Jiangsu Province 210029, PR China
[b] Department of Toxicology, School of Public Health, Nanjing Medical University, Hanzhong Road 140, Nanjing, Jiangsu Province 210029, PR China

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ABSTRACT

Although flunarizine (FLN) has been widely used for migraine prophylaxis with clear success, the mechanisms of its actions in migraine prophylaxis are not completely understood. It has been hypothesized that migraine is a channelopathy, and abnormal activities of voltage-gated Na^+ and Ca^{2+} channels might represent a potential mechanism of cortical hyperexcitability predisposing to migraine. The aim of the present study was to investigate the effects of FLN on Na^+ and Ca^{2+} channels of cultured rat cortical neurons. Sodium currents (I_{Na}) and calcium currents (I_{Ca}) in cultured rat cortical neurons were monitored using whole-cell patch-clamp recordings. Both I_{Na} and I_{Ca} were blocked by FLN in a concentration-dependent manner with IC_{50} values of 0.94 μM and 1.77 μM, respectively. The blockade of I_{Na} was more powerful at more depolarizing holding potentials. The steady-state inactivation curve of I_{Na} was shifted towards more hyperpolarizing potentials by FLN. FLN significantly delayed the recovery from fast inactivation of I_{Na}. Furthermore, the action of FLN in blocking I_{Na} was enhanced at higher rates of channel activation. Blockades of these currents might help explain the mechanism underlying the preventive effect of FLN on migraine attacks.

Migraine is a common episodic brain disorder affecting more than 10% of the general population; it is typically characterized by recurrent attacks of disabling headaches and associated symptoms. Cortical spreading depression (CSD) represents a pathophysiological signal that has been associated with the induction of migraine. In particular, it can represent an electrical correlate of the neurological symptoms in migraine with aura [11]. CSD is a wave of neuronal excitation that slowly propagates across the cortex, causing brief intense spike trains followed by long-lasting suppression of neuronal activities [6]. The mechanism of this ictal phenomenon involves ionic channels and many neurotransmitter systems. Alterations at these levels could create a condition of cortical hyperexcitability, which could increase susceptibility to CSD [2]. Interestingly, abnormal activities of either voltage-gated Na^+ or Ca^{2+} channels have been reported in some forms of migraine [10]. Migraine could be considered as a channelopathy, and abnormal activities of Na^+ and Ca^{2+} channels might represent a potential mechanism of cortical hyperexcitability predisposing to migraine [12,19].

FLN, a lipophilic diphenylpiperazine derivative, has been widely used for migraine prophylaxis with clear success. The mechanism of its action in migraine prophylaxis is not completely understood, but it may include the reduction of neural NO synthase activity, the blockade of 5-HT release and inhibition of neurovascular inflammation [13]. As mentioned above, it may also include cortical hyperexcitability due to abnormal activities of Na^+ and Ca^{2+} channels. In the present study, electrophysiological experiments were undertaken to analyze the effects of FLN on Na^+ and Ca^{2+} channels in cultured rat cortical neurons.

All experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Neurons were cultured from the cortex of E17-18 fetal rats as described [1]. Neurons were maintained in neurobasal medium supplemented with 2% B27, 100 U ml^{-1} penicillin, and 100 μg ml^{-1} streptomycin (half of the volume replaced every 3 days), and used for electrophysiological experiments between day 7 and 10.

An EPC-9 amplifier and PULSE 8.02 software (HEKA Elektronik, Germany) were used. Currents were recorded from cortical neurons with pyramidal morphology and relatively short processes. Patch pipettes were pulled from thin-walled borosilicate glass on a vertical puller (Narishige Scientific Instrument Laboratory, Tokyo, Japan; model PP-83) and had a resistance of 2–5 MΩ. The internal solution for recording of I_{Na} contained (in mM): 140 CsF, 10 NaCl, 1 EGTA, 10 HEPES, 2 Mg-ATP, and the pH was adjusted to 7.3 with CsOH. The internal solution for recording of I_{Ca} contained (in mM): 125 CsCl, 2 $MgCl_2$, 20 HEPES, 11 EGTA, 1 $CaCl_2$, 4.5 Mg-ATP, 0.3 Li-GTP, and the pH was adjusted to 7.3 with CsOH. The extracellular solution for recording of I_{Na} contained (in mM): 120 NaCl, 20 TEA-Cl, 5 CsCl,

* Corresponding author. Tel.: +86 02583714511; fax: +86 02583714511.
E-mail address: chinaqiwan@126.com (Q. Wan).

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1 $MgCl_2$, 1 $CaCl_2$, 0.1 $CdCl_2$, 10 HEPES, 10 D-glucose, and the pH was adjusted to 7.3 with NaOH. The extracellular solution for recording of I_{Ca} contained (in mM): 140 TEA-Cl, 5 $CaCl_2$, 0.8 $MgCl_2$, 10 HEPES, 11 D-glucose, and the pH was adjusted to 7.3 with Tris-base. Capacity transients were cancelled and series resistance compensated (>70%) by use of the internal circuitry of EPC-9. After the establishment of the whole-cell condition, currents were allowed to stabilize for a few minutes before starting data acquisition. All experiments were performed at room temperature.

Patch-clamp recording results obtained from different treatments of cortical neurons were analyzed using PULSEFIT software (Heka Elektronik, Germany). The concentration–response curves were fitted to the Hill equation: $I/I_{max} = 1/[1 + (C/IC_{50})^H]$, where I and I_{max} are the observed and maximum blocking percentages of current, C is the concentration of the drug, and IC_{50} is the concentration producing a half-maximal current block. Steady-state inactivation curves were fitted to the Boltzmann equation: $I/I_{max} = 1 + \exp[(V_m - V_{i1/2})/k_i]$, where I_{max} is the maximum of peak currents, V_m is the prepulse potential, and $V_{i1/2}$ and k_i the voltage for half-inactivation potential and the slope factor, respectively. Data are expressed as means ± S.E.M. for all the experiments. Paired Student's t-test was used as appropriate to evaluate the statistical significance of differences between two group means. All tests of statistical significance were two-sided and the level of statistical significance was set at $P < 0.05$.

FLN (Sigma–Aldrich, St. Louis, MO, USA) was prepared as a 100 mM stock solution in dimethylsulfoxide (DMSO) and diluted in the extracellular solution for each experiment to give the desired final concentration just before use. Other chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA). Cell culture media and supplements were from Invitrogen (Grand Island, NY, USA).

To study the effects of FLN on I_{Na}, cells were depolarized for 25 ms from the holding potential of −80 mV to various potentials ranging from −80 mV to +40 mV (5 mV steps) every 1 s. The inward currents were completely blocked by perfusion of 1 μM tetrodotoxin (TTX) and then defined as TTX-sensitive Na^+ currents. After application of 1 μM FLN in bath solution, the peak amplitudes of I_{Na} were decreased immediately (Fig. 1A). The threshold for activation was shifted from −55 mV to −60 mV. The potential at which I_{Na} reached a maximum was shifted from −30 mV to −40 mV (Fig. 1B).

The concentration-dependent effects of FLN on I_{Na} were tested by superfusing cortical neurons with different concentrations of FLN. Current recordings were obtained before and 3 min after superfusion of the cells with each concentration of the drug. FLN at 0.1, 1, 10, and 100 μM markedly blocked the peak amplitudes of

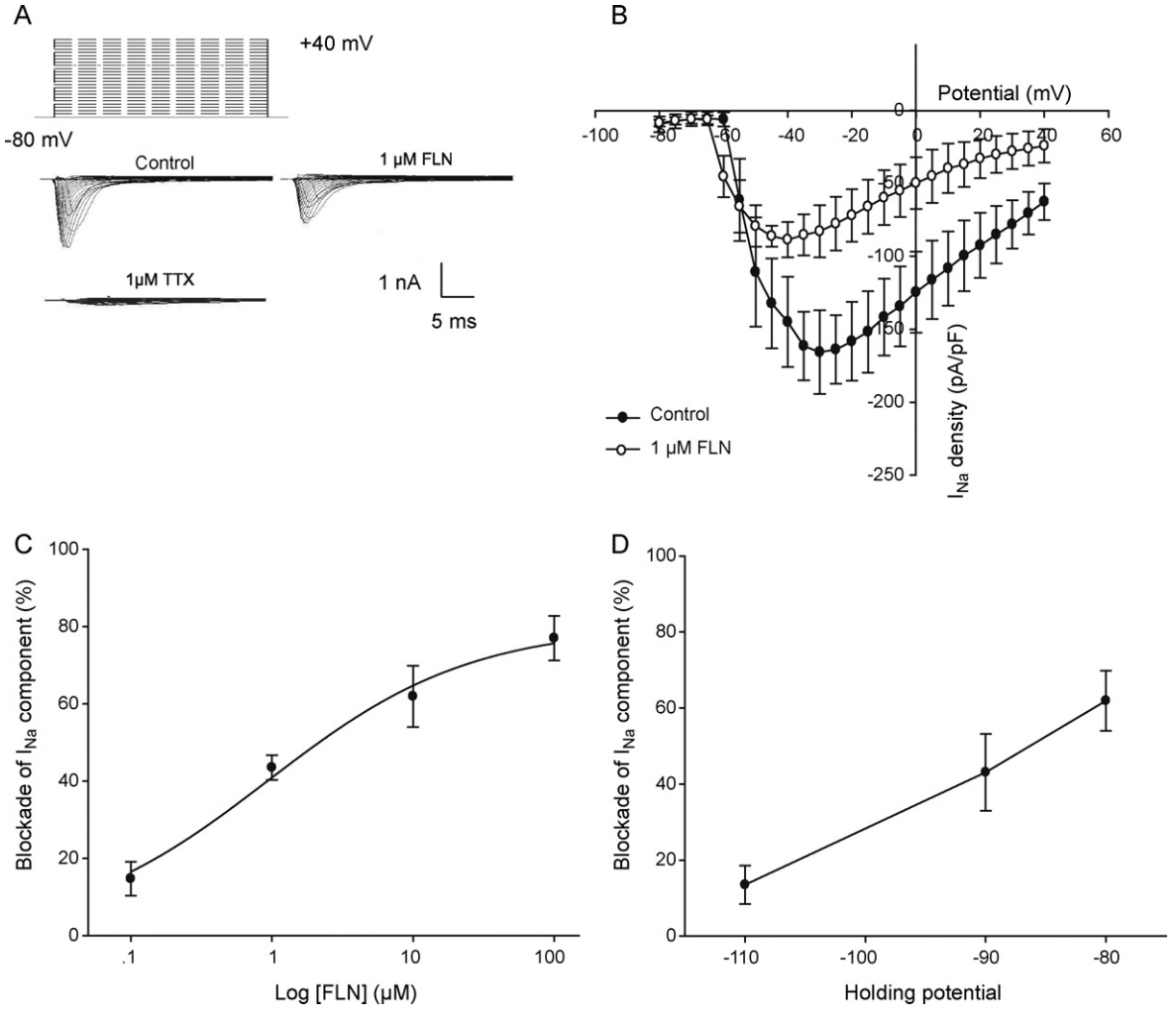

Fig. 1. Effects of FLN on I_{Na} in cultured rat cortical neurons. (A) Depolarizing steps from −80 mV to +40 mV (5 mV steps) from a holding potential of −80 mV induced inward currents that were blocked by 1 μM perfusion of TTX. The peak amplitudes of I_{Na} were decreased immediately after application of 1 μM FLN in bath solution. (B) I–V relationship for the mean of the normalized peak currents evoked between −80 and +40 mV in 5 mV increments in control condition and during FLN perfusion (n = 8). (C) Concentration-dependent effects of FLN on I_{Na}. (D) Voltage-dependent effects of FLN on I_{Na}. Cells were held at −110, −90 or −80 mV and FLN was applied during a 3 min period in all cases.