Mechanism of Sodium Channel Block by Venlafaxine in Guinea Pig Ventricular Myocytes

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ABSTRACT

Venlafaxine is a newly introduced antidepressant agent. The drug causes selective inhibition of neuronal reuptake of serotonin and norepinephrine with little effect on other neurotransmitter systems. Cases of seizures, tachycardia, and QRS prolongation have been observed following drug overdose in humans. The clinical manifestations of cardiac toxicity suggest that venlafaxine may exhibit cardiac electrophysiological effects on fast conducting cells. Consequently, studies were undertaken to characterize effects of venlafaxine on the fast inward sodium current (INa) of isolated guinea pig ventricular myocytes. Currents were recorded with the whole-cell configuration of the patch-clamp technique in the presence of Ca2+ and K+ channel blockers. Results obtained demonstrated that venlafaxine inhibits peak INa in a concentration-dependent manner with an estimated IC50 of 8 · 10−6 M. Inhibition was exclusively of a tonic nature and rate-independent. Neither kinetics of inactivation (τinac = 0.652 ± 0.020 ms, under control conditions; τinac = 0.636 ± 0.050, in the presence of 10−6 M venlafaxine; n = 5 cells isolated from five animals) nor kinetics of recovery from inactivation of the sodium channels (τrec = 58.7 ± 1.6 ms, under control conditions; τrec = 54.4 ± 1.8, in the presence of 10−5 M venlafaxine; n = 10 cells isolated from six animals) were significantly altered by 10−5 M venlafaxine. These observations led us to conclude that venlafaxine blocks INa following its binding to the resting state of the channel. Thus, the characteristics of block of INa by venlafaxine are different from those usually observed with most tricyclic antidepressants or conventional class I antiarrhythmic drugs.

Depression is a very common and disabling disease with important social and economic implications (Wells et al., 1989). Although many antidepressant agents are available for the management of this disorder, their efficacy is often limited because toxic events can be encountered. Their most serious adverse effects include the risk for life-threatening arrhythmias, especially in patients with preexisting cardiac disease or after overdose administration. Newly introduced antidepressive agents, including venlafaxine (Effexor), have improved compliance and response to drug treatment in various psychiatric disorders (Thase, 1996). Indeed, venlafaxine is perceived as a first-line agent in the treatment of depression because the drug possesses a better safety profile than some older antidepressant agents (Cunningham et al., 1994; Rudolph and Derivan, 1996; Ballenger, 1996; Van Gelder et al., 1998). Pharmacological action of venlafaxine involves inhibition of presynaptic reuptake of serotonin, norepinephrine, and, to a lesser extent, dopamine. Unlike the tricyclic antidepressant agents, venlafaxine does not have affinity for histamine, muscarinic, or α1-adrenergic receptors (Grunder et al., 1996). Thus, it should not exhibit side effects traditionally associated with blockade of these receptors.

Despite several studies evaluating venlafaxine efficacy and side effect profiles, there is limited information on its electrophysiological cardiovascular properties. Treatment with venlafaxine could be associated with some cardiovascular effects such as elevation of heart rate and blood pressure (Grunder et al., 1996; Rudolph and Derivan, 1996; Khan, 1998). Cases of seizures, hypotension, and sinus tachycardia have also been described in the setting of overdose administration (Fantaskey and Burkhart, 1995; Parsons et al., 1996; Dahl et al., 1996; Durback and Scharman, 1996; Kokan and Hart, 1996; Zhalkovsky et al., 1997; Peano et al., 1997; Rosen et al., 1997). In addition, widening of the QRS interval has been observed during treatment with venlafaxine in some patients. This observation led us to examine the cardiac electrophysiological properties of the drug by studying its effects on inward sodium current (INa), the major ionic current involved in the depolarization of cardiac ventricular myocytes and impulse propagation within the ventricles.

ABBREVIATION: INa, sodium current.
Materials and Methods

Experiments were performed in accordance with institutional guidelines of Laval University on animal use in research. Animals were housed and maintained in compliance with the Guide to the Care and Use of Experimental Animals of the Canadian Council on Animal Care.

Cell Preparation and Electrophysiological Measurements. Experiments were performed on single ventricular myocytes obtained from adult guinea pig hearts by use of an enzymatic dissociation technique described in Daleau et al. (1995). A small aliquot of dissociated cells was placed in a 0.5-ml chamber mounted on the stage of an inverted microscope (model CK2; Olympus). Cells were allowed to adhere to the coverslip at the bottom of the chamber and were superfused continuously with the external solution containing 20 mM NaCl, 110 mM CsCl, 2 mM MgCl2, 2 mM CaCl2, 3 mM CoCl2, 10 mM HEPES, 10 mM glucose, and 10 mM tetraethylammonium. The presence of cobalt in the extracellular solution is expected to cause a 5-mV negative shift in INa availability (Hanck and Sheets, 1992a). The pH of the extracellular solution was adjusted to 7.35 with CsOH. Venlafaxine was added to the extracellular solution to cause a 5-mV negative shift in INa availability (Hanck and Sheets, 1992a). The pH of the extracellular solution was adjusted to 7.35 with CsOH. Venlafaxine was added to the extracellular solution to obtain desired final drug concentrations (10⁻⁶ M, 3 · 10⁻⁶ M, 10⁻⁵ M, 3 · 10⁻⁵ M, and 10⁻⁴ M). The temperature of the perfusate was kept at room temperature (21–23°C). In our experiments, steady-state drug effects were observed within 2 to 3 min when the superfusion rate was 2 ml/min. Patch-clamp pipette electrodes were pulled from glass capillary tubes (1.2 mm o.d.; Radnoti Glass Technology, Inc., Monrovia, CA) on a microelectrode puller (model P-87; Sutter Instruments Co., Novato, CA) and heat-polished with a microforge. Pipettes had tip resistances between 0.5 and 1.0 MΩ and heat-polished with a microforge. Pipettes had tip resistances between 0.5 and 1.0 MΩ when filled with the intracellular solution containing 110 mM CsF, 10 mM NaF, 30 mM CsCl, 10 mM HEPES, 2 mM EGTA, 2 mM MgCl2, and 5 mM K₂ATP. The pH was adjusted to 7.35 with CsOH. All currents were recorded in the whole-cell, voltage-clamp configuration of the patch-clamp technique with an Axopatch-1D amplifier (Axon Instruments, Inc., Burlingame, CA). Voltage-clamp command pulses were generated by a 12-bit digital-to-analog converter (model TL/1; Axon Instruments, Inc.) controlled by the PCLAMP software package (version 5.7.1; Axon Instruments, Inc.). Rod-shaped cells with clear cross striations, resting potentials more negative than −78 mV, and stable sodium current (as assessed during a baseline period of at least 4 min) were used. Cells with Na⁺ currents >10 nA were rejected.

Voltage-clamp protocols are illustrated in the insets of Figs. 1–6 and are described in the respective figure legends. Cells were maintained at a holding potential of −120 mV between pulse protocols to allow full recovery from inactivation. The peak amplitude of INa in all cells (n = 27 from 15 animals) exposed to the drug. Figure 1 illustrates a typical example of sodium current recordings elicited by 30-ms pulses to −20 mV from a holding potential of −120 mV under control conditions, and appropriate fittings showed no significant changes in kinetics of inactivation of the currents (τinac = 0.652 ± 0.020 ms, under control conditions; τinac = 0.636 ± 0.050, in the presence of venlafaxine 10⁻⁵ M; n = 5 cells isolated from five animals). Decrease in INa was concentration-dependent with an estimated IC₅₀ of 8 · 10⁻⁶ M (Fig. 2).

Statistics. Data are expressed as means ± S.E. Statistical significance (P < .05) of difference between two means was judged with paired or unpaired Student’s t test as appropriate.

Results

Experiments performed in isolated guinea pig ventricular myocytes with the patch-clamp technique demonstrated that venlafaxine (10⁻⁶–10⁻⁴ M) caused a significant decrease in peak INa in all cells (n = 27 from 15 animals) exposed to the drug. Figure 1 illustrates a typical example of sodium current recordings elicited by 30-ms pulses to −20 mV from a holding potential of −120 mV under control conditions, in the presence of venlafaxine 10⁻⁵ M and after 5-min washout. A progressive near complete recovery of current was observed after removal of the drug. Sodium currents recorded in the presence of venlafaxine were normalized to that recorded under control conditions, and appropriate fittings showed no significant changes in kinetics of inactivation of the currents (τinac = 0.652 ± 0.020 ms, under control conditions; τinac = 0.636 ± 0.050, in the presence of venlafaxine 10⁻⁵ M; n = 5 cells isolated from five animals). Decrease in INa was concentration-dependent with an estimated IC₅₀ of 8 · 10⁻⁶ M (Fig. 2).

To elucidate the mechanism of action of venlafaxine on cardiac sodium current, we performed additional protocols exploring modifications of INa characteristics in the presence of the drug at a concentration of 10⁻⁵ M (≈IC₅₀) and compared current elicited under control conditions to that observed in the presence of the drug.

![Fig. 1. Recordings of sodium currents elicited by 30 ms pulses to −20 mV from a holding potential of −20 mV under control conditions, in the presence of 10⁻⁶ M venlafaxine and after washout of the drug.](image-url)
**Time Course of Block Development.** Block induced by venlafaxine $10^{-5}$ M was assessed with 8-Hz trains (30 pulses; 30-ms pulse duration). At each pulse, cells were depolarized from a holding potential of $-120$ mV to a test potential of $-20$ mV. In the absence of the drug, repetitive pulsing did not affect $I_{Na}$ magnitude. As well, in the presence of venlafaxine, maximal reduction of the current was reached instantaneously, showing block with no use-dependent characteristics. Similar results were found with 2.5-Hz trains of depolarizing pulses from different holding potentials to the same test potential of $-20$ mV (Fig. 3). In addition, block of $I_{Na}$ was rate-independent regardless of the holding potential tested ($-120$, $-130$, and $-140$ mV). Thus, no phasic block of $I_{Na}$ could be detected, venlafaxine causing only a tonic block on cardiac sodium current.

**Kinetics of Recovery from Inactivation.** Time course of recovery from sodium channel inactivation was characterized with the double-pulse protocol shown in the inset of Fig. 4. Steady-state sodium channel inactivation was achieved with a 100-ms conditioning pulse to $-20$ mV, and the time course of recovery was determined with a test pulse (30 ms to $-20$ mV from a holding potential of $-120$ mV) at different coupling intervals, ranging from 2 to 4000 ms. In Fig. 4, the peak current measured following each test pulse was normalized to matching control values and plotted as a function of the recovery time. Under control conditions as well as in the presence of venlafaxine, reactivation of $I_{Na}$ at $-120$ mV was monoeXponential. Time constants were $58.7 \pm 1.6$ ms and $54.4 \pm 1.8$ ms ($n = 10$ cells isolated from six animals) under control conditions and in the presence of venlafaxine $10^{-5}$ M, respectively. These data suggest that venlafaxine reduces the amplitude of $I_{Na}$ without altering the time course of recovery from sodium channel inactivation.

**Steady-State Inactivation of Sodium Channel.** Sodium currents were measured during a 30-ms test pulse to $-20$ mV after a 2000-ms depolarization to different potentials varying between $-140$ and $-30$ mV (Fig. 5). Examples of sodium currents recorded in the absence and presence of venlafaxine ($10^{-5}$ M) are presented in Fig. 5A. The experimental values were well fit to a Boltzmann equation (Fig. 5B). Under control conditions, the mean values for $E_{1/2}$ and $k$ averaged $-76 \pm 2$ mV and $4.9 \pm 0.2$ mV, respectively ($n = 10$ cells isolated from six guinea pigs). Venlafaxine ($10^{-5}$ M) reduced the maximum available $I_{Na}$ by $51 \pm 9\%$ and significantly shifted the midpoint by $10 \pm 2$ mV toward more negative potentials ($-86 \pm 2$ mV, $n = 10$ isolated from six guinea pigs, $p < .05$). No significant changes in the slope factor of the inactivation curve were observed in presence of venlafaxine ($5.5 \pm 0.3$ mV, $n = 10$ isolated from six guinea pigs, $p > .05$).

**Discussion**

Results obtained in this study demonstrate that venlafaxine is a relatively potent cardiac sodium channel blocker. Inhibition of $I_{Na}$ by venlafaxine was concentration-dependent with an estimated IC$_{50}$ of $8 \times 10^{-6}$ M. Block of $I_{Na}$ by venlafaxine was essentially tonic; no use-dependent characteristics could be detected. Moreover, venlafaxine reduced $I_{Na}$ current without affecting the time course of recovery from sodium channel inactivation. These observations led us to conclude that venlafaxine blocks $I_{Na}$ by binding to the resting state of the channel.

One of the important observations made during this study was the demonstration that venlafaxine inhibits $I_{Na}$ by $\approx 50\%$ at a concentration of $10^{-5}$ M and also shifts the midpoint of $I_{Na}$ availability by $-10$ mV. Previous studies have reported a shift toward the negative potentials by $-0.41$ mV/min in the half-point of $I_{Na}$ availability with time (Hanck and Sheets, 1992b). This effect could not account for the entire shift caused by venlafaxine because effects were observed within 5 to 10 min upon exposure to the drug. Incomplete recovery of drug effects on $I_{Na}$ amplitude during the washout period could be partially explained by this effect.

Results were reproduced in all cells tested. Although statistical analysis was realized on the number of cells (total number, 27), we suggest that the number of animals from which these cells were isolated (total number, 15) is enough to consider this sample size as adequate.

Venlafaxine has been shown to be effective in the treat-
normalization of the currents in the presence of venlafaxine. A clear shift with solid lines (B). The dotted line represents the curve fit following isolated from six guinea pigs).

About 5 to 10% of the white population have an autosomal recessive trait for impaired ability to metabolize drugs via enzyme CYP2D6 and are described poor metabolizers (Brosen, 1990; DeVane, 1994; Hamelin et al., 1996), could provoke accumulation of the drug and predispose patients to proarrhythmia.

Block of sodium current by venlafaxine appears to be different from that described with tricyclic antidepressant agents and type I antiarrhythmic agents. Indeed, block of sodium current is often comprised of both tonic and use-dependent characteristics (Schaufler et al., 1975; Muir et al., 1982; Hondeghem, 1987; Kojima et al., 1989; Ogata and Narahashi, 1989; Krafte et al., 1994). In contrast, our results demonstrated that venlafaxine blocks sodium current in a use-independent manner. In addition, block did not change with hyperpolarization in the range of voltages tested. These observations led us to conclude that venlafaxine blocks $I_{\text{Na}}$ in its resting state. Finally, venlafaxine did not affect the time of recovery from sodium channel inactivation. Voltage dependence of channel availability was in full agreement with tonic block observed during previous experiments.

In conclusion, our results demonstrate that venlafaxine possesses direct cardiac electrophysiological effects by inhibiting $I_{\text{Na}}$ in ventricular myocytes. Although an extrapolation of data obtained from animal experiments to human must be taken with care, we speculate that reported effects of venlafaxine could explain QRS prolongation and proarrhythmia observed in some patients with increased plasma concentrations of the drug. We propose that better knowledge of the potential cardiac electrophysiological effects of venlafaxine may result in a safer use of the drug.

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References


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