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# AJMALINE-INDUCED BLOCK OF SODIUM CURRENT IN RAT VENTRICULAR MYOCYTES

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A b s t r a c t

The aim of the present study was to explore concentration-, voltage- and frequency-dependence of the sodium current ($I_{Na}$) block induced by ajmaline in enzymatically isolated rat ventricular myocytes. Voltage clamp experiments in a whole-cell patch-clamp arrangement were performed on 32 cells. The blocking effect was evaluated in the range of concentrations between 0.3 and 5000 μmol/l at room temperature. The level of block was concentration-dependent and the effect was fully reversible. The results of pooled data analysis (18 cells and 25 measurements) showed the values of $EC_{50}$ and the Hill coefficient to be 8.2 ± 1.5 μmol/l and 0.62 ± 0.08, respectively. The voltage- and frequency-dependence of the drug effect was analysed at 3 μmol/l. The block appeared to be voltage-dependent but frequency-independent in contrast to propafenone (0.2 μmol/l) reported previously. An analysis of the rate of block level variations in response to voltage steps revealed substantial differences between kinetics of the block development in both drugs, which corresponded well with the observed difference in the frequency response.

K e y w o r d s

Antiarrhythmic, Ajmaline, Sodium current, Rat, Patch-clamp technique

## INTRODUCTION

Ajmaline is a highly effective antiarrhythmic drug usually included in class Ia according to the Vaughan-Williams classification (*1*). It has been used for the treatment of various types of both atrial and ventricular tachyarrhythmias in clinical practice for over forty years. In the medical treatment of sustained ventricular tachycardia, ajmaline was reported to be more effective than lidocaine (*2*). Ajmaline has been used to convert atrial fibrillation to sinus rhythm and also in the treatment of patients with Wolf-Parkinson-White syndrome with paroxysmal atrial fibrillation because of its pronounced effect on the accessory pathways (*1, 3, 4)*. Recently, ajmaline has been used to unmask the concealed or intermittent forms of Brugada syndrome caused by mutation or polymorphism in the sodium cardiac channel gene (*5, 6*). Electrophysiological studies have indicated that ajmaline slows down conduction in atrial and ventricular myocardium and prolongs refractory periods (*7, 8*). Besides the well-known induction of a block of the fast sodium current $I_{Na}$, ajmaline also blocks the L-type

calcium current $I_{Ca\text{-}L}$, inward moiety of potassium current $I_{K1}$ and delayed rectifier potassium current $I_K$ in guinea-pig ventricular myocytes (*9*).

Early studies on multicellular cardiac preparations demonstrated that the ajmaline-induced block of $I_{Na}$ changes the raising phase of the action potential $(dV/dt)_{max}$. In stimulated preparations, ajmaline decelerated the phase of fast depolarisation (*10*). If the stimulation was discontinued, depolarisation was the faster the longer was the break *(11)*. Also clinically used derivatives of ajmaline, namely prajmalium and detajmium, were the objects of several electrophysiological studies. The blocking effects of prajmalium (N-propyl derivative of ajmaline) on $I_{Na}$ in isolated rat (*12*), frog (*13*) and rabbit (*14*) cardiomyocytes were explored and the concentration-, use- and frequency-dependence of the effect was described. The effect of detajmium (4,3'-diethylamino-2'-hydroxypropyl-ajmaline) was reported to be frequency-dependent in isolated dog ventricular muscle and Purkinje fibres when the conventional intracellular microelectrode technique was applied (*15*). The frequency-dependent block of $I_{Na}$ induced by most of the class I antiarrhythmic drugs is of clinical importance because it implies a more effective suppression of premature than regular excitations.

According to our knowledge, the effect of ajmaline on $I_{Na}$ has not been studied in isolated cardiac myocytes so far. The aim of our experiments was to evaluate concentration- and frequency-dependence of the ajmaline-induced block of $I_{Na}$ in rat ventricular cardiomyocytes. The results were compared with previously studied effects of propafenone (*16*).

## MATERIALS AND METHODS

### Cell isolation

The experiments were performed on enzymatically isolated rat ventricular myocytes (adult male Wistar rats, 250 ± 50 g). The animals were sacrificed by cervical dislocation under mild ether anaesthesia. The heart was removed and placed into an ice-cold Krebs-Henseleit solution. Subsequently, the heart was attached to a Langendorff apparatus and retrogradely perfused through the aorta. Perfusion with a 0.9 mmol/l $CaCl_2$ Tyrode solution, lasting 3 to 5 min, was followed by application of a nominally Ca-free Tyrode solution (for up to 4.5 min). Collagenase (type I; Sigma, 0.32 mg/ml) and protease (type XIV; Sigma, 0.053 mg/ml) were added to calcium-free Tyrode solution to obtain the first enzyme solution (4 min exposure). The second enzyme solution differed from the first one by the absence of protease (15 min exposure). The enzyme solution was then washed out by two low calcium Tyrode solutions (0.09 and 0.18 mmol/l $CaCl_2$). All solutions were oxygenated with 100% $O_2$ at 37 °C.

Both ventricles were then cut into small pieces with fine scissors in 30 ml of a 0.18 mmol/l $CaCl_2$ Tyrode solution to produce a suspension. This was filtered and 20 ml of the Tyrode solution containing 1.8 mmol/l $CaCl_2$ was added gradually within 20 min to achieve a $Ca^{2+}$ concentration of 0.9 mmol/l. The whole procedure of myocyte isolation was carried out at 37 °C.

### Solutions

The composition of calcium-free Tyrode solution (in mmol/l) was as follows: NaCl, 135; KCl, 5.4; $MgCl_2$, 0.9; HEPES, 10; $NaH_2PO_4$, 0.33; glucose, 10 (pH was adjusted to 7.4 with NaOH). The

patch electrode filling solution contained (mmol/l): K-aspartate, 130; KCl, 25; $MgCl_2$, 1; $Na_2ATP$, 5; EGTA, 1; HEPES, 5; GTP, 0.1 (pH at 7.25 adjusted with KOH).

WHOLE-CELL VOLTAGE CLAMP

Single rod-shape cells with a well apparent striation were used for membrane current recordings by the whole-cell patch-clamp technique. Filled glass electrodes with a low resistance of about 1 MΩ were selected to keep the access resistance as low as possible. For generation of voltage clamp protocols and data acquisition, the Axopatch 200A equipment (Axon Instruments, Inc.) and pCLAMP programme (version 6.0.4) with a personal computer were used. The experiments were performed at room temperature (between 19 and 27 °C) under control conditions and in the presence of ajmaline (Gilurytmal$^R$10, Solvay Pharmaceuticals) applied at concentrations between 0.3 μmol/l and 5 mmol/l. For comparison, some experiments were repeated under the effect of 0.2 μmol/l propafenone.

The analysis was based on an assumption that, under the drug effect, the total number of channels responsible for $I_{Na}$ consisted of a fraction of channels blocked by the drug (non-conductive) and the remaining channels not influenced by the drug (unblocked channels). Then the fraction of unblocked channels (Funbl) was estimated as the ratio of peak $I_{Na}$ in the presence of the drug to the peak $I_{Na}$ under control conditions. The fraction of blocked channels (Fbl) was expressed as 1-Funbl.

## RESULTS

The concentration-dependence of the effect of ajmaline on $I_{Na}$ (*Fig. 1*) was measured at regular stimulation (1 Hz) by rectangular pulses (200 ms, 60 mV); the holding voltage was - 80 mV. The peak values of $I_{Na}$ were evaluated at the steady state, as shown in the inset of *Fig. 1*. Ajmaline at concentrations between $10^{-6}$ and $5\times10^{-3}$ mol/l suppressed $I_{Na}$ in a concentration-dependent manner. The fraction of unblocked channels (Funbl) was estimated as the ratio of the drug-affected current to the current recorded under control conditions. To quantify the blocking effects, the pooled data were approximated (for the best fit) by the Hill equation

$$F_{unbl} = \frac{(EC_{50})^n}{c^n + (EC_{50})^n}$$

where c denotes the concentration of ajmaline, $EC_{50}$ is the concentration required for half-maximal inhibition and n is the Hill coefficient. The resulting values of $EC_{50}$ and the Hill coefficient for $I_{Na}$ were 8.2 ± 1.5 μmol/l and 0.62 ± 0.08 respectively. The blocking effects of ajmaline were reversible at all concentrations used (*Fig. 2*).

The frequency-dependence of the ajmaline-induced $I_{Na}$-block was tested by 200 ms pulses from -80 mV to –20 mV at regular steady-state stimulation. The peak value of $I_{Na}$ decreased slightly with increasing frequency both under control conditions and after the addition of ajmaline (not shown). However, their ratio (regarded as an approximate indicator of the fraction of unblocked channels) appeared to be frequency-independent in contrast to a significant frequency-dependent block revealed in experiments with propafenone (*Fig. 3*). To explain

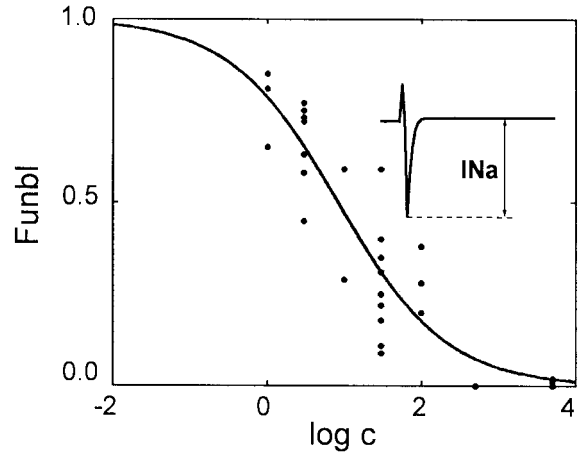


*Fig. 1*
Concentration-dependence of the blocking effect of ajmaline on $I_{Na}$. Fractions of unblocked channels (Funbl) are plotted against the common logarithm of concentration c [µmol/l]. Pooled data from 18 cells are approximated by a theoretical function (full line). Inset: reading of $I_{Na}$; current trace was recorded in response to an imposed pulse from -75 to -30 mV.

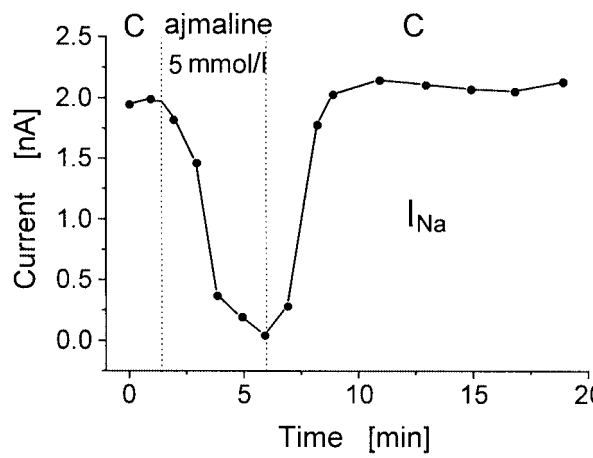


*Fig. 2*
Time course of the onset and offset of an $I_{Na}$-block due to administration and washout of ajmaline (5 mmol/l). C, control solution.

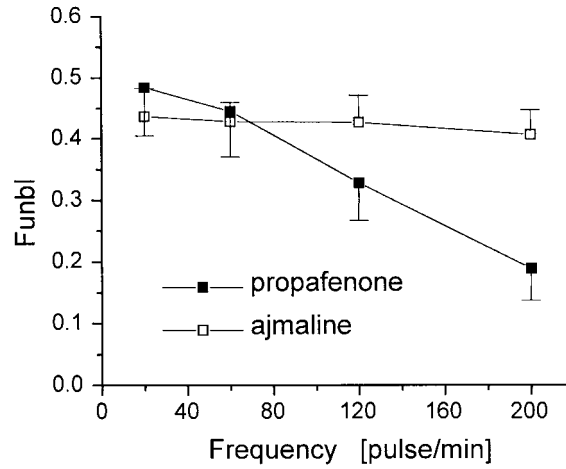


*Fig. 3*
The frequency-dependence of an $I_{Na}$-block induced by ajmaline (3 µmol/l) or propafenone (0.2 µmol/l). Mean values ± SE from four experiments are shown.

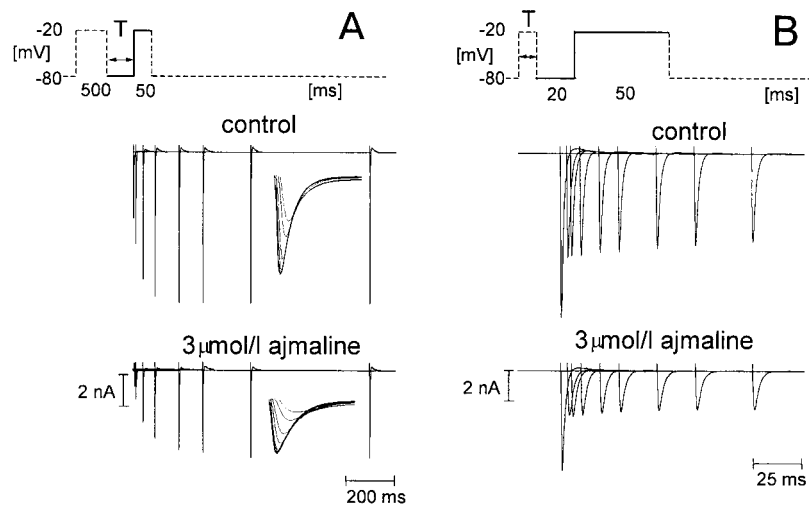


*Fig. 4*
A: Recovery of $I_{Na}$ from inactivation and from the block induced by a standard conditioning pulse (500 ms) in a representative experiment. The resting interval T between the conditioning and test pulses was progressively prolonged. Original traces are responses of $I_{Na}$ to test pulses.
B: Development of an $I_{Na}$-block during depolarising steps. Duration of the conditioning depolarising pulse T (separated from the test pulse by a constant rest interval of 20 ms) was progressively prolonged (first trace T = 0 ms).

this difference, we analysed the development of $I_{Na}$-block separately during depolarization to -20 mV and during the rest interval at -80 mV.

*Fig. 4A* shows the original records of $I_{Na}$, as affected by progressively prolonged rest intervals T between the conditioning and the test pulses under control conditions and after addition of 3 μmol/l ajmaline. While, under control conditions, changes in $I_{Na}$ reflected pure recovery of $I_{Na}$ from inactivation, in the presence of ajmaline, they included an additional voltage-dependent component related to the drug effect.

Similarly, to visualise changes in the block during depolarisation, the duration of a single conditioning pulse was gradually increased (T in *Fig. 4B*). During a 20-ms long rest interval between the conditioning and the test pulse, $I_{Na}$ partly recovered from inactivation. The test $I_{Na}$ did not depend significantly on a progressive prolongation of T, except for T = 0 ms, both in control conditions and in the presence of ajmaline.

The experimental data illustrated in *Fig. 4* were used to construct the time course of $I_{Na}$-block during and after a depolarising pulse (*Fig. 5*). The block exhibited voltage-dependence in both drugs. There was, however, a striking difference in the rate of equilibration of the block level. The ajmaline-induced block approached its altered level very fast, i.e., within milliseconds or tenths of a millisecond. On the other hand, changes in the propafenone-induced block invoked by both depolarising and repolarising steps proceeded in two phases. A slow phase following a fast one faded out during several hundreds of milliseconds.

## DISCUSSION

In the present study, the class I antiarrhythmic agent ajmaline suppressed the fast sodium current $I_{Na}$ in a concentration-dependent manner characterised by an $EC^{50}$ of 8.2 ± 1.5 μmol/l and a Hill coefficient of 0.62 ± 0.08. We also calculated $EC^{50}$ from the previously published values of $(dV/dt)_{max}$-depression in multi-cellular cardiac preparations (*7*). The obtained value of 7.65 ± 1.0 μmol/l was very close to our own data and to the value of 6.6 μmol/l obtained in isolated skeletal muscle fibres assessed by direct measurement of $I_{Na}$, as reported by *Körper et al.* (*17*). For comparison, measurements of the effect of an ajmaline derivative (N-n propylajmaline – prajmalium) resulted in an $EC_{50}$ of 3 μmol/l (*14*) and a Hill coefficient of 0.7 (*18*). The former study was carried out on rabbit ventricular myocytes with the maximum upstroke velocity $(dV/dt)_{max}$ as an $I_{Na}$ indicator, the latter on DPI-modified Na-channels of neonatal rat cardiocytes. The fact that the $EC_{50}$ value for $I_{Na}$-block induced by prajmalium was lower than that induced by ajmaline confirms the higher effectiveness of prajmalium in comparison with ajmaline, as found by experiments performed on an isolated left atrium of the guinea-pig (*19*).

The majority of antiarrhythmic drugs with the main effect on $I_{Na}$, e.g., amiodarone, flecainide, lidocaine, mexiletine, procainamide and propafenone,

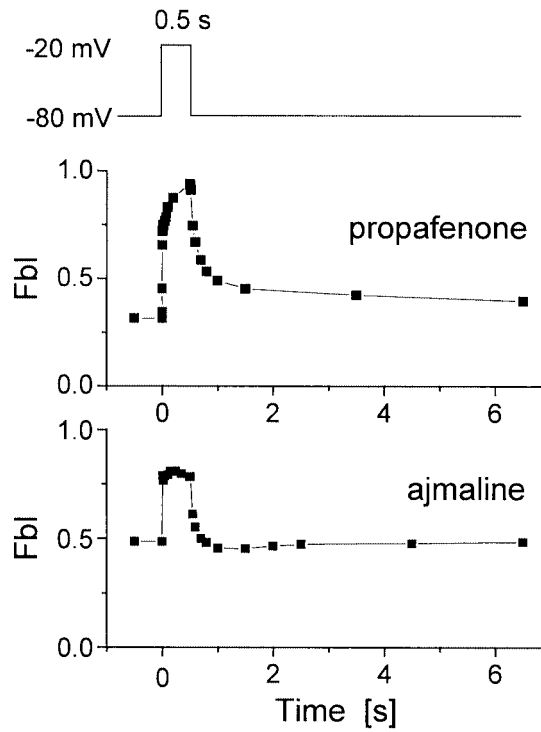


*Fig. 5*
Comparison of constructed variations of the level of $I_{Na}$-block during and after a depolarising pulse under the effect of either propafenone (0.2 μmol/l) or ajmaline (3 μmol/l).

have been shown to exhibit a voltage- and frequency-dependent block (*20*). In the present study, the voltage- and frequency-dependence of ajmaline effects on $I_{Na}$ was analysed and compared with the properties of a propafenone block. Some features of the propafenone-induced block are also described in our previous study (*16*). While the propafenone-induced block increased significantly with stimulation frequency, the effect of ajmaline appeared to be frequency-independent up to 200 p. min. (*Fig. 2*). The explanation of this difference follows from the reconstruction of block development in the course of an imposed rectangular pulse of membrane voltage (*Fig. 5*). The level of block is voltage-dependent in both cases. The time course of a response of the propafenone-induced block to both depolarising and repolarising steps of membrane voltage may be approximated by two exponentials corresponding to fast and slow processes. In the

case of ajmaline, the slow processes during a 6-second interval were missing (*Fig. 5*). This might explain the frequency-independence of the ajmaline-induced block in the range of stimulation periods between 0.3 and 3.0 s (*Fig. 3*).

*Kodama et al. (21)* analysed the dependence of block levels (characterised by $(dV/dt)_{max}$) on the duration of a preceding conditioning voltage-clamped pulse under the effects of six representatives of class I anti-arrhythmic drugs. They distinguished two types of $I_{Na}$-blocks. While, in the first type, the $(dV/dt)_{max}$ reduction enhanced progressively as the clamp pulse duration was prolonged, in the second type, the significant reduction in $(dV/dt)_{max}$ at the shortest clamp pulse was followed by a small additional progressive decay. Our results suggest that the propafenone-induced block represents the second type of this classification. At the shortest conditioning pulses, ajmaline produced a significant increase in the block, which can be interpreted as an open-channel block. However, the additional progressive decay, interpreted as an inactivated channel block, was missing.

A c k n o w l e d g e m e n t s

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BLOKÁDA SODÍKOVÉHO PROUDU INDUKOVANÁ AJMALINEM U KOMOROVÝCH KARDIOMYOCYTŮ POTKANA

S o u h r n

Cílem této studie bylo prozkoumat koncentrační, napěťovou a frekvenční závislost blokády sodíkového proudu ($I_{Na}$) indukované ajmalinem u enzymaticky izolovaných komorových kardiomyocytů potkana. Experimenty byly provedeny metodou vnuceného napětí na 32 buňkách. Blokující efekt byl hodnocen v rozmezí koncentrací 0,3 až 5000 μmol/l při pokojové teplotě. Hloubka blokády závisela na koncentraci ajmalinu a účinek byl zcela reverzibilní. Analýza získaných dat (18 buněk a 25 měření) vedla ke stanovení parametru $EC_{50}$ 8,2 ± 1,5 μmol/l a Hillova koeficientu 0,62 ± 0,08. Napěťová a frekvenční závislost byla vyhodnocována při koncentraci 3 μmol/l. Účinek ajmalinu byl napěťově závislý, ale na rozdíl od dříve studovaného propafenonu (0,2 μmol/l) frekvenčně nezávislý. Analýza časových změn hloubky blokády v odpovědi na napěťové impulsy odhalila podstatné rozdíly v kinetice rozvoje blokády u obou látek ve shodě s pozorovaným rozdílem ve frekvenční závislosti.

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