Effect of Ajmaline on Action Potential and Ionic Currents in Rat Ventricular Myocytes

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Abstract. The effect of ajmaline on action potential (AP) and ionic current components has been investigated in right ventricular myocytes of rat at room temperature using the whole cell patch clamp technique. Ajmaline decreased the upstroke velocity ($(\mathrm{d}V/\mathrm{d}t)_{\max}$) of AP and the AP amplitude, increased the AP duration measured at 50 and 90 % repolarization, and reversibly inhibited most components of membrane ionic current in a concentration-dependent manner. The following values of IC_{50} and of the Hill coefficient (n_{H}) resulted from approximation of the measured data by the Hill formula: for fast sodium current (I_{Na}) $IC_{50} = 27.8 \pm 1.14$ μmol/l and $n_{\mathrm{H}} = 1.27 \pm 0.25$ at holding potential -75 mV, $IC_{50} = 47.2 \pm 1.16$ μmol/l and $n_{\mathrm{H}} = 1.16 \pm 0.21$ at holding potential -120 mV; for L-type calcium current ($I_{\mathrm{Ca\text{-}L}}$) $IC_{50} = 70.8 \pm 0.09$ μmol/l and $n_{\mathrm{H}} = 0.99 \pm 0.09$; for transient outward potassium current (I_{to}) $IC_{50} = 25.9 \pm 2.91$ μmol/l and $n_{\mathrm{H}} = 1.07 \pm 0.15$; for ATP-sensitive potassium current ($I_{\mathrm{K(ATP)}}$) $IC_{50} = 13.3 \pm 1.1$ μmol/l and $n_{\mathrm{H}} = 1.16 \pm 0.15$. The current measured at the end of 300 ms depolarizing impulse was composed of an ajmaline-insensitive component and a component inhibited with $IC_{50} = 61.0 \pm 1.1$ μmol/l and $n_{\mathrm{H}} = 0.91 \pm 0.08$. At hyperpolarizing voltages, ajmaline at high concentration of 300 μmol/l reduced the inward moiety of time-independent potassium current (I_{K1}) by 36 %. The results indicate that the inhibition of I_{Na} causes both the decreased rate of rise of depolarizing phase and the lowered amplitude of AP. The inhibition of I_{to} is responsible for the ajmaline-induced AP prolongation.

Key words: Ajmaline — Action potential — Ionic currents — Concentration dependence — Rat ventricular myocytes

Introduction

Ajmaline (Fig. 1) is a highly effective antiarrhythmic drug usually classified as Ia according to Vaughan-Williams classification (Ito et al. 1990; Schuchert and Meinertz 2000). It has been used for treatment of various types of both atrial and

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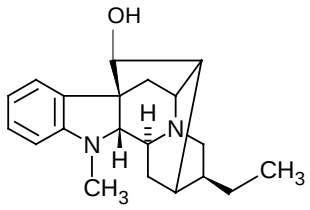

Figure 1. Structure of ajmaline (modified from Körper et al. 1998).

ventricular tachyarrhythmias for over thirty years. In the treatment of sustained ventricular tachycardia, ajmaline was reported to be more effective than lidocaine (Manz et al. 1992). 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 (Ito et al. 1990; Manz and Luderitz 1993; Chen et al. 1994; Dagres et al. 2000). Padrini et al. (1993) demonstrated deceleration of atrioventricular conduction in men. Recently, ajmaline and other antiarrhythmic drugs that inhibit fast sodium current (I_{Na}) have been proven useful for diagnosis of the concealed or intermittent forms of the Brugada and LQT-3 syndromes (Eckardt et al.1999; Brugada et al. 2000; Liu et al. 2002).

Early experimental studies have demonstrated the main electrophysiological effect of ajmaline, deceleration of conduction in atrial and ventricular myocardium that corresponded to a decrease of upstroke velocity ($(\mathrm{d}V/\mathrm{d}t)_{\mathrm{max}}$) of action potential (AP) (Heistracher 1964, 1971; Liebeswar et al. 1970; Bojorges et al. 1975; Arribas et al. 1986). The direct effect of ajmaline on I_{Na} was investigated in our earlier work (Bahníková et al. 2002).

Ajmaline (as well as other Ia antiarrhythmic drugs) prolonged the refractory period in cardiac tissue as a consequence of AP prolongation (Bojorges et al. 1975; Arribas et al. 1986). Voltage clamp studies of the effect of ajmaline on the currents that may form the repolarization phase of AP are, however, sparse. The effect on the transient outward potassium current (I_{to}) as well as on the ATP-sensitive potassium current ($I_{\mathrm{K(ATP)}}$) was described in cloned cardiac channels expressed in *Xenopus* oocytes (Sakuta et al. 1992; Rolf et al. 2000). Enomoto et al. (1995) demonstrated the ajmaline-induced block of delayed rectifier potassium current (I_{K}), inward moiety of time-independent potassium current (I_{K1}) and L-type calcium current ($I_{\mathrm{Ca\text{-}L}}$) in guinea-pig ventricular myocytes. Kiesecker et al. (2004) demonstrated the ajmaline-induced block of the HERG (human ether a-go-go related gene) channel, a structural correlate of the rapid component of I_{K}, expressed in human embryonic kidney cells and in *Xenopus* oocytes. The effect of ajmaline on I_{to} in cardiomyocytes was first described in our previous work (Bébarová et al. 2005).

This study was aimed to determine the concentration-dependent effects of ajmaline on depolarizing and repolarizing ionic current components responsible for the changes in AP configuration in rat ventricular myocytes.

Materials and Methods

Cell isolation

Ventricular myocytes were isolated from right hearts of adult male Wistar rats (250 ± 50 g). The dissociation procedure has been previously described in detail (Bébarová et al. 2005). In brief, the heart was retrogradely perfused *via* aorta with 0.9 mmol/l $CaCl_2$ Tyrode solution and then with nominally Ca-free Tyrode solution. During the first digestion step, the perfusion was continued with nominally Ca-free Tyrode solution containing collagenase type S (0.2 mg/ml; Yakult), protease type XIV (0.053 mg/ml; Sigma), albumin bovine fraction V (2 mg/ml; Sigma), and EGTA (44 μmol/l; Sigma). In the second digestion step, protease and albumin in the enzyme solution were omitted. The enzyme solution was then washed out in two steps by perfusion with low calcium Tyrode solutions (0.09 and 0.18 mmol/l $CaCl_2$). All solutions were oxygenated with 100 % O_2 at 37 ℃.

The experiments were carried out in accordance with the institutional guidelines and approved by the local authorities (permit No. 076/2001-V3).

Solutions and chemicals

Composition of the Tyrode solution was following (mmol/l): NaCl 135, KCl 5.4, $CaCl_2$ 0.9, $MgCl_2$ 0.9, HEPES 10, NaH_2PO_4 0.33, glucose 10 (pH was adjusted to 7.4 with NaOH). To block I_{Ca} and I_{to} currents, $CoCl_2$ (2 mmol/l) and 4-aminopyridine (3 mmol/l), respectively, were added. $I_{K(ATP)}$ was evoked in the presence of 200 μmol/l dinitrophenol.

The patch electrode filling solution used for the measurement of I_{Na} and I_{Ca} contained (mmol/l): L-aspartic acid 130, TEACl 25, $MgCl_2$ 1, Na_2ATP 5, EGTA 1, HEPES 5, GTP 0.1 (pH 7.25 adjusted with CsOH); the patch electrode filling solution used for the measurement of potassium currents contained (mmol/l): L-aspartic acid 130, KCl 25, $MgCl_2$ 1, Na_2ATP 5, EGTA 1, HEPES 5, GTP 0.1, Na_2-phosphocreatine 3 (pH 7.25 adjusted with KOH).

$CoCl_2$ (Sigma) and 4-aminopyridine (Sigma) were dissolved in deionized water to obtain stock solutions (1 mol/l and 100 mmol/l, respectively). In the case of 4-aminopyridine, pH was adjusted to 7.4 with HCl. 2,4-dinitrophenol (Sigma) was prepared as 10 mmol/l stock solution in ethanol. Ajmaline (Gilurytmal® 10, Solvay Pharmaceuticals) was applied in concentrations ranging between 0.3 μmol/l and 5 mmol/l.

Electrophysiological measurements

Single rod-shaped cells with well apparent striations were used for the membrane voltage and current recordings applying the whole cell patch clamp technique. The patch pipettes were pulled from borosilicate glass capillary tubes and heat polished on a programmable horizontal puller (Zeitz-Instrumente). The resistance of the filled glass electrodes was below 1.5 MΩ to keep the access resistance as low as possible. For generation of experimental protocols and data acquisition,

the Axopatch 200A equipment (Axon Instruments, Inc.) and pCLAMP program (version 6.0.4) were used. The currents were digitally sampled at 4 kHz and stored on the hard disk of a computer. Experiments were performed at room temperature.

In the current clamp experiments, APs were recorded at regular stimulation (0.2 Hz) in control conditions (Tyrode solution) and in the presence of ajmaline (30 μmol/l). Concentration dependence of the ajmaline effect on the ionic currents was established at 0.1 Hz. Voltage-clamped rectangular pulses were used for measurement of all components except of the time-independent $I_{K(ATP)}$ which was evaluated at the level of 0 mV from the responses to the imposed 200 ms lasting ramp pulses (from +120 to −120 mV). Since the rate of apparent inactivation of I_{to} was strongly affected by ajmaline, the time integral of the current was used as a measure of blockade instead of the current amplitude. Experimental protocols are described in Results.

Statistical analysis

Results are presented as means ± S.E. The curve fitting and the paired *t*-test (used to assess the significance of the effects of ajmaline with the drug-free conditions as control) were performed using GraphPad Prism, version 4.0 (GraphPad Software, Inc.).

Results

Effect of ajmaline on AP configuration

Fig. 2 shows superimposed APs recorded in control and under the effect of 30 μmol/l ajmaline. The drug-affected AP exhibited reduced amplitude and increased

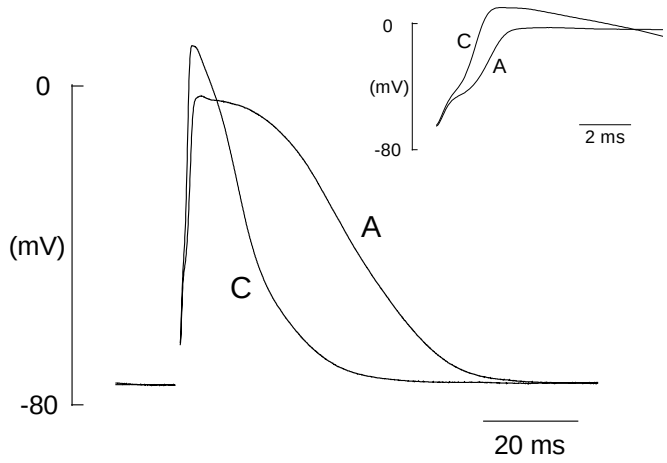

Figure 2. Effect of ajmaline (30 μmol/l) on AP configuration at stimulating frequency of 0.2 Hz. Inset: detail of the first 10 ms. C, control; A, ajmaline.

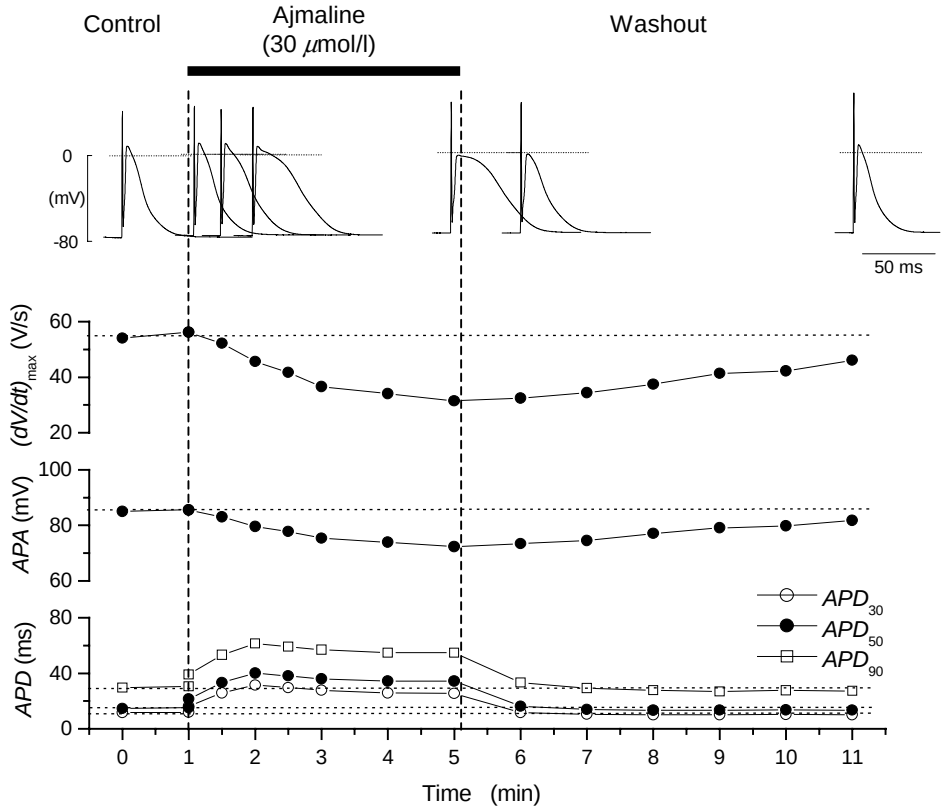

Figure 3. Changes of AP configuration after application and washout of ajmaline (30 μmol/l). Frequency of stimulation was 0.2 Hz. $(\mathrm{d}V/\mathrm{d}t)_{\max}$, upstroke velocity; *APA*, AP amplitude; APD_{30}, APD_{50} and APD_{90}, AP duration at the level of 30 %, 50 % and 90 % repolarization (related to control record). The top panel illustrates selected original courses of AP during experiment.

duration so that both traces intersected. The upstroke velocity of AP was substantially reduced (inset). At lower concentrations ajmaline induced similar but less pronounced changes of AP configuration (not shown).

The effect of ajmaline was reversible. Its development in a representative experiment is illustrated in Fig. 3. AP duration measured at 30, 50 and 90 % repolarization of the control AP (APD_{30}, APD_{50} and APD_{90}) achieved the new steady state rapidly both after application and after washing out of ajmaline. The effect on $(\mathrm{d}V/\mathrm{d}t)_{\max}$ and AP amplitude developed and faded out more slowly.

The results of measurements in five cells are summarized in Table 1. The parameter APD_{30} was omitted. At 30 % repolarization, the factors responsible for suppression of AP amplitude and for the increase of AP duration counteracted re-

Table 1. Parameters of the AP waveform (mean ± S.E., $n = 5$) in control conditions and under the effect of ajmaline (30 μmol/l)

	Control	Ajmaline (30 μmol/l)
RMP (mV)	−72.6 ± 0.9	−72.2 ± 1.0*
$(\mathrm{d}V/\mathrm{d}t)_{\max}$ (V/s)	68.2 ± 12.9	33.7 ± 13.5*
APA (mV)	92.9 ± 6.6	74.8 ± 3.2**
APD_{50} (ms)	13.1 ± 1.0	31.9 ± 6.1*
APD_{90} (ms)	30.8 ± 4.6	65.9 ± 12.9*

RMP, resting membrane potential; $(\mathrm{d}V/\mathrm{d}t)_{\max}$, upstroke velocity; APA, AP amplitude; APD_{50} and APD_{90}, AP duration at 50 and 90 % of repolarization (related to control record). * $p < 0.05$, ** $p < 0.01$.

sulting in large dispersion of APD_{30} values. In the extreme, AP amplitude decreased even below 30 % of the control so that APD_{30} could not be assessed. The relatively high variability of AP prolongation correlated with variable ratio of $I_{\text{Ca-L}}$ to I_{to} observed in voltage clamp experiments in individual cells. The resting membrane voltage was slightly shifted towards depolarization.

Effect of ajmaline on ionic currents

Ajmaline inhibited all main components of membrane ionic current in a concentration-dependent manner (Fig. 4 and Fig. 5). The blocking effects were reversible at all concentrations applied (not illustrated). The superimposed original records (left panels) illustrate the effect of ajmaline at 30 μmol/l and the way of reading the current components. Using the least square best fit procedure, the pooled data (right panels) could be well approximated by the Hill equation

$$\frac{I_{\text{ajmaline}}}{I_{\text{control}}} = \frac{(IC_{50})^{n_{\text{H}}}}{[D]^{n_{\text{H}}} + (IC_{50})^{n_{\text{H}}}} \tag{1}$$

The ratio of currents recorded in presence of ajmaline and in control conditions represents the fraction of unblocked channels related to individual current components. $[D]$ stands for drug concentration, IC_{50} and n_{H} denote the concentration required for half-maximal inhibition of the current and n_{H}, respectively.

The effects of ajmaline on depolarizing inward currents (I_{Na} and $I_{\text{Ca-L}}$) are illustrated in Fig. 4. Potassium currents were partially blocked by tetraethylammonium (TEA) and Cs^+ in the pipette solution. Ajmaline suppressed the current amplitudes but did not significantly influence the time course of inactivation as documented by overlapping of currents normalized to peak values (insets).

I_{Na} was measured in the presence of Co^{2+} (2 mmol/l) as the difference between the peak current and the current at the end of 50 ms test pulse from the holding potential −75 mV to +40 mV. The relatively high level of positive voltage was used to minimise current amplitude and thus the error due to series access resistance. If a 100 ms hyperpolarizing prepulse to −120 mV was applied, the inhibition of I_{Na}

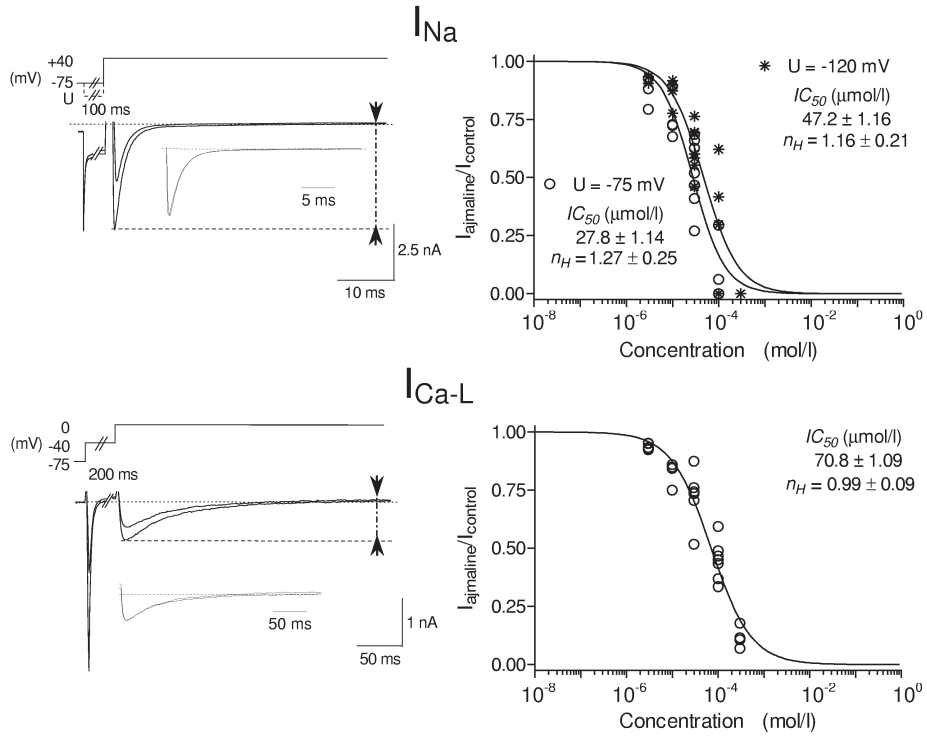

Figure 4. Effect of ajmaline on depolarizing ionic currents. Left panels: experimental protocols and superimposed original records of the sodium current (I_{Na}) and the L-type calcium current ($I_{\mathrm{Ca\text{-}L}}$) in control conditions and in the presence of ajmaline (30 μmol/l). Arrowheads indicate the way of reading currents. Dotted lines indicate zero current levels. Insets: superimposed currents normalized to peak values. Note different current and time scales. Right panels: concentration dependence of the effect of ajmaline. Two curves in the case of I_{Na} illustrate the effect of hyperpolarizing pulse ($U = -120$ mV) on concentration-dependent inhibition. IC_{50} and the Hill coefficient (n_{H}) resulting from the measured data are indicated.

decreased. This became manifest as a shift of the concentration dependence curve to the right and of the increase of IC_{50} from 27.8 to 47.2 μmol/l. In both cases, the fitting procedure resulted in n_{H} slightly exceeding unity.

$I_{\mathrm{Ca\text{-}L}}$ was measured at 0 mV as the difference between the peak current and the current at the end of 300 ms test pulse preceded by 200 ms depolarizing prepulse to -40 mV to inactivate I_{Na}. The best-fit procedure resulted in $IC_{50} = 70.8 \pm 1.09$ μmol/l; n_{H} was close to unity.

Concentration-response curve was plotted for three types of outward current (Fig. 5): I_{to}, current measured at the end of 300 ms depolarizing pulse ($I_{\mathrm{K,end}}$), and $I_{\mathrm{K(ATP)}}$.

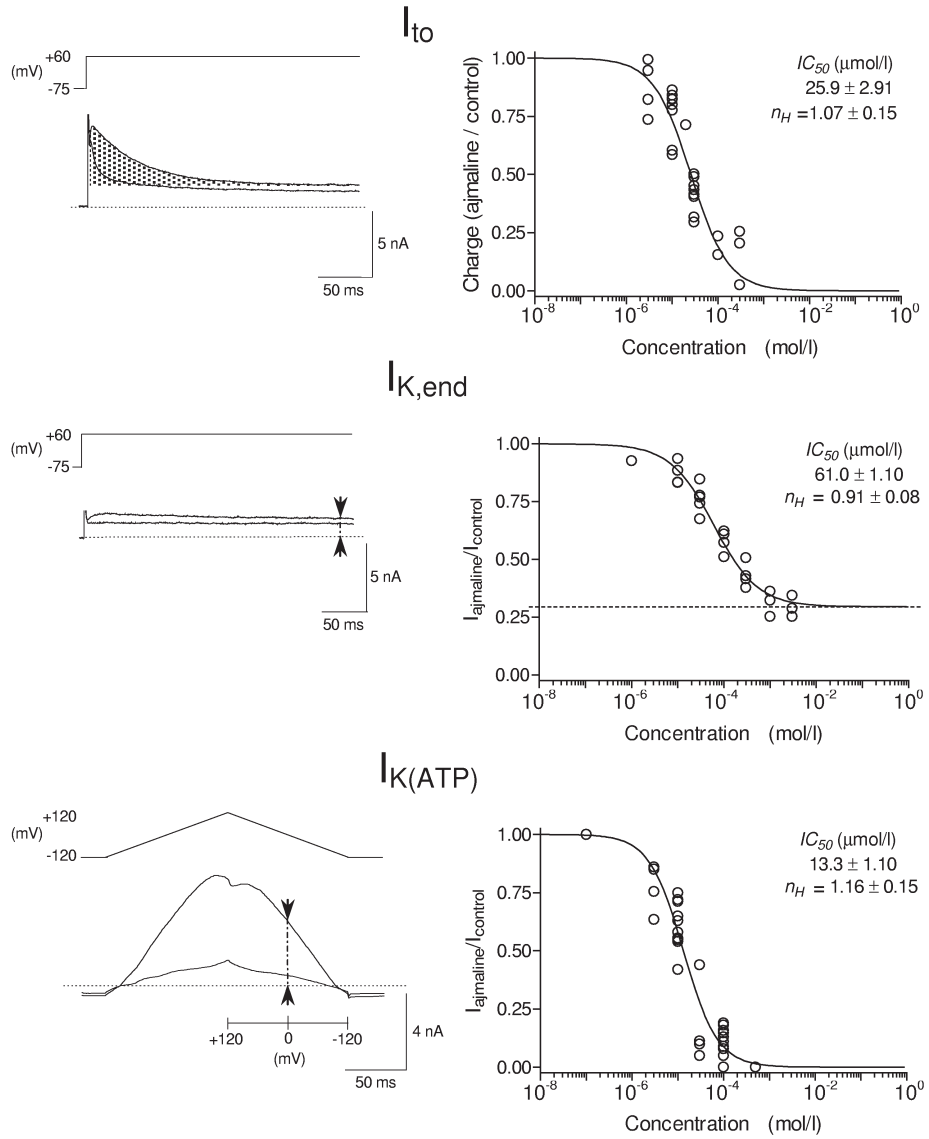

Figure 5. Effect of ajmaline on repolarizing potassium currents. Left panels: experimental protocols and superimposed original records of the transient outward potassium current (I_{to}), the current measured at the end of 300 ms depolarizing pulse ($I_{\mathrm{K,end}}$) and the ATP-sensitive potassium current ($I_{\mathrm{K(ATP)}}$) evoked by dinitrophenol (200 μmol/l) before and after addition of ajmaline (30 μmol/l) to extracellular solution. Dotted lines indicate zero current levels, arrowheads indicate the way of reading currents. I_{to} was represented by its time integral (dotted area). Right panels: concentration dependence of the effect of ajmaline. IC_{50} and the Hill coefficient (n_{H}) resulting from the measured data are indicated. About 30% of $I_{\mathrm{K,end}}$ was ajmaline-insensitive.

I_{to} was recorded in response to 300 ms pulses from −75 to +60 mV while $I_{Ca\text{-}L}$ was blocked by Co^{2+} (2 mmol/l). Since the rate of apparent inactivation of I_{to} increased considerably with the concentration of ajmaline, we regarded the time integral of the current a better measure of blockade than the current amplitude. The constant current component measured at the end of the depolarizing pulse was subtracted. To eliminate the capacity current, the lower limit of integration was shifted by 1 ms from the onset of the pulse.

$I_{K,end}$ measured in presence of 4-aminopyridine (3 mmol/l) and Co^{2+} (2 mmol/l) at the end of 300 ms depolarizing impulse was not fully blocked even at high concentrations above 1 mmol/l indicating an involvement of at least two components, one being ajmaline-insensitive.

$I_{K(ATP)}$ was recorded in response to 200 ms lasting ramp pulses from +120 to −120 mV after activation by the metabolic inhibitor dinitrophenol (200 μmol/l) and the current recorded in the absence of dinitrophenol was subtracted therefrom. To detect $I_{K(ATP)}$, the descendent part of the imposed ramp was used so as to prevent contamination by I_{Na}. The inhibition of $I_{K(ATP)}$ was voltage-independent (not shown). For concentration-response analysis, $I_{K(ATP)}$ was evaluated at 0 mV. Surprisingly, comparing the values of IC_{50}, $I_{K(ATP)}$ appeared to be even more sensitive to ajmaline than I_{Na}.

In four experiments, the effect of ajmaline on the inward rectifier I_{K1} was examined at hyperpolarizing voltages. Considering its very low sensitivity for the drug, only the effect at 300 μmol/l was tested. I_{K1} was reduced by $36.05 \pm 7.62\,\%$ (not illustrated) and this inhibition in the examined voltage range between −135 and −75 mV was voltage-independent.

The values of IC_{50} and n_H of all explored currents are summarized in Table 2. The sensitivity of current components to ajmaline, aligned according to IC_{50} values, declined in the following order: $I_{K(ATP)} - I_{to}$ and $I_{Na} - I_{K,end} - I_{Ca\text{-}L}$; n_H did not significantly differ from unity.

Table 2. Values of the concentration required for half-maximal inhibition of the current (IC_{50}) and Hill coefficient (n_H) for the ajmaline-induced block of ionic membrane currents in rat ventricular myocytes

Current	IC_{50} (μmol/l)	n_H
I_{Na}	27.8 ± 1.14	1.27 ± 0.25
$I_{Ca\text{-}L}$	70.8 ± 1.09	0.99 ± 0.09
I_{to}	25.9 ± 2.91	1.07 ± 0.15
$I_{K,end}$	61.0 ± 1.10	0.91 ± 0.08
$I_{K(ATP)}$	13.3 ± 1.10	1.16 ± 0.15

I_{Na}, sodium current; $I_{Ca\text{-}L}$, L-type calcium current; I_{to}, transient outward potassium current; $I_{K,end}$, the current measured at the end of 300 ms depolarizing pulse; $I_{K(ATP)}$, ATP-sensitive potassium current.

Discussion

Efffect of ajmaline on AP

To avoid considerable variability in the configuration of the APs due to regional heterogeneity in rat ventricles (Clark et al. 1993; Shimoni et al. 1994), we restricted our experiments to the cells from right ventricles only.

In the presence of ajmaline, we observed marked decrease in $(\mathrm{d}V/\mathrm{d}t)_{\max}$ of AP, decline of the AP amplitude and the AP prolongation. Similar changes of AP configuration after administration of ajmaline have been described previously in various experimental models. A decrease in $(\mathrm{d}V/\mathrm{d}t)_{\max}$ and amplitude of the AP, as well as an increase in the duration of the AP were observed in dog atrial and ventricular muscle (Bojorges et al. 1975), in guinea pig ventricular myocytes (Enomoto et al. 1995) and also in men (Eckardt et al. 1999).

Effect of ajmaline on ionic currents

The main purpose of this study was to assess the effect of ajmaline on ionic current components (I_{Na}, $I_{\mathrm{Ca\text{-}L}}$, I_{to}, I_{K}, I_{K1}, and $I_{\mathrm{K(ATP)}}$) under comparable experimental conditions.

In early studies, the sensitivity of I_{Na} to ajmaline was examined indirectly using $(\mathrm{d}V/\mathrm{d}t)_{\max}$ as indicator. In Purkinje fibres, ajmaline in concentration 15 μmol/l suppressed $(\mathrm{d}V/\mathrm{d}t)_{\max}$ by about 67 % (Heistracher 1964) and in concentration 10 μmol/l by about 57 % (Obayashi and Mandel 1976). The direct effect of ajmaline on I_{Na} was first studied in our earlier work (Bahníková et al. 2002). Approximation of the measured data with the Hill equation led to the value of $IC_{50} = 8.2 \pm 1.5$ μmol/l. In the present series of experiments, the IC_{50} was surprisingly higher (27.8 ± 1.1 μmol/l). The former measurements were carried out on cells from left and right ventricles and the data dispersion was considerably higher than in the present series restricted to right ventricles.

The blocking effect of ajmaline on I_{Na} depended on holding voltage (Fig. 4) in agreement with the effect on $(\mathrm{d}V/\mathrm{d}t)_{\max}$ as described by Heistracher (1964). This property of I_{Na}-block were demonstrated also under the effect of other antiarrhythmic agents, e.g. bidisomide in rat ventricular myocytes (Homma et al. 2001) and moricizine in guinea-pig atrial myocytes (Ahmmed et al. 2002). The shift of the concentration-response relationship to higher concentrations at more negative holding voltages indicates that the block developed primarily in the inactivated channel state. The degree of block may decline at hyperpolarized voltages as a consequence of a decreased fraction of inactivated sodium channels.

The effect of ajmaline on $I_{\mathrm{Ca\text{-}L}}$ was reported previously only by Enomoto et al. (1995). In the guinea-pig ventricular myocytes, they set the value of the apparent dissociation constant K_{D} at 12 μmol/l. In our experiments we arrived at $IC_{50} = 70.8$ μmol/l. Such a discrepancy can hardly be attributed only to species differences. Unfortunately, no comparable data measured in rat cardiomyocytes are available. Besides longer test pulses (300 ms *vs.* 100 ms used by Enomoto et

al. 1995) the experimental conditions differed mainly in suppression of ajmaline-sensitive potassium currents in our experiments.

The sensitivity of I_{to} to ajmaline was relatively high as previously demonstrated and discussed in detail in our recent study (Bébarová et al. 2005). In contrast to I_{Na}-block, the concentration dependence of I_{to}-block appeared to be insensitive to variations of holding voltage (explored by 2 s lasting conditioning pulses separated from the test pulse by 150 ms at −75 mV – not illustrated).

The sustained current measured at the end of depolarizing pulse in rat ventricular myocytes is composed of more components that have so far not been definitely interpreted (Apkon and Nerbonne 1991; Slawsky and Castle 1994; Scamps 1996; Sanchez-Chapula 1999). Confining our estimation of the ajmaline effect on the total sustained current, we found that only a part of this current was ajmaline-sensitive.

In our study, the effect of ajmaline on dinitrophenol-activated $I_{K(ATP)}$ was measured on the descending part of a ramp impulse at the voltage level of 0 mV to minimize contamination by other ionic currents (Christé et al. 1999). Not surprisingly, the resulting IC_{50} (13.3 μmol/l) was much lower than IC_{50} assessed in cloned cardiac channels expressed in *Xenopus* oocytes (145 μmol/l, Sakuta et al. 1992). From the published data, it appears that as much as tenfold concentration of an antiarrhythmic drug is needed to obtain an effect comparable with that in mammalian cells (Rolf et al. 2000).

Ionic currents underlying modulation of AP

In our experimental conditions, ajmaline inhibited all principal ionic membrane currents. According to IC_{50}, the sensitivity to the ajmaline-induced block declined in the following order: $I_{K(ATP)}$ (13.3 μmol/l) – I_{to} (25.2 μmol/l) and I_{Na} (27.8 μmol/l) – $I_{K,end}$ (61.0 μmol/l) – $I_{Ca\text{-}L}$ (70.8 μmol/l). Under normal experimental conditions, $I_{K(ATP)}$ is negligible so that I_{to} and I_{Na} seem to be the main candidates to explain the observed effect of ajmaline on AP configuration (Fig. 2).

The inhibition of I_{Na} is responsible for reduction of $(dV/dt)_{max}$, and very likely also for the decline of AP amplitude because it was manifested even in the presence of $I_{Ca\text{-}L}$ blocker (2 mmol/l Co^{2+}, not shown). This view is consistent with the effect of tetrodotoxin (TTX) on AP configuration and I_{Na} components in the rat: Abraham et al. (1989) observed the decrease in $(dV/dt)_{max}$ and the reduction of AP amplitude, and Saint et al. (1992) demonstrated two distinct TTX-sensitive components of I_{Na}, a transient and a persistent one. They suggested that the persistent current might contribute to the plateau phase of AP in the rat. The same authors (Ju et al. 1992) have shown that besides TTX, lignocaine (class I antiarrhythmic agent) blocked the persistent I_{Na} and concomitantly affected the plateau phase of AP. The persistent current was blocked by lignocaine even in lower concentration than the transient I_{Na}.

The inhibition of I_{to} as a principle repolarizing current in the rat (Apkon and Nerbonne 1991) is obviously the main determinant of ajmaline-induced AP prolongation. The effect on I_{to} apparently prevails over the effect of $I_{Ca\text{-}L}$ inhibition that would lead rather to AP shortening. The sensitivity of I_{to} to ajmaline estimated

in this work is very likely related mainly to its fast component because in control, the time course of inactivation could be well fitted by a single exponential function with the time constant of around 37 ms (Bébarová et al. 2005). Although I_{to} was found to be composed of both fast and slow components even in right ventricular free wall of rat, the fast component prevailed (Wickenden et al. 1999).

The contribution of I_K and I_{K1} to the formation of AP configuration in rat cardiomyocytes is regarded very small (Apkon and Nerbonne 1991; Varro et al. 1993). Thus, significant participation of these components in the effect of ajmaline on AP is unlikely.

Clinical relevance

The high sensitivity of potassium currents (particularly I_{to} and $I_{K(ATP)}$) demonstrated in this study may be of clinical significance. The inhibition of potassium currents and the consequent increase in AP duration can have beneficial but also adverse effects. The prolonged refractory period increases drug efficiency in suppressing reentry arrhythmias. On the other hand, deteriorated balance between inward and potassium outward currents during AP repolarization may lead to the development of early afterdepolarizations, ventricular tachyarrhythmia of the torsades de pointes type (e.g. Hii et al. 1992; Roden 1993; Hohnloser and Singh 1995; Haverkamp et al. 2001) which can even degenerate into ventricular fibrilation (Cubeddu 2003). Thus, in some clinical situations, ajmaline must be applied with caution. In the presence of structural heart disease, particularly if associated with ventricular dysfunction, there is a significant risk of ventricular arrhythmia (Schrickel et al. 2003). Recently, the role of the genetic diseases of potassium channels in congenital long QT syndrome has been largely explained (for review, see Roden 2003). In these cases, the application of ajmaline would be hazardous.

The high sensitivity of $I_{K(ATP)}$ to ajmaline might explain the high effectivity of this antiarrhythmic drug in the treatment of ventricular arrhythmia in the acute phase of myocardial infarction resistant to conventional doses of lidocaine (Grenadier et al. 1983). On the other hand, the block of $I_{K(ATP)}$ may be responsible for the arrhythmogenic action of ajmaline in patients with ischemic heart disease because ATP-sensitive potassium channels play an important role in protection against ischemic damage of the heart (for reviews, see Šimurdová and Bravený 1998; Gross and Peart 2003; Kolář and Ošťádal 2004).

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