USE-DEPENDENT BLOCK AND RECOVERY OF NA^+ CHANNELS BY CLASS IC ANTIARRHYTHMIC DRUGS (FLECAINIDE AND ETHACIZIN) IN CANINE VENTRICULAR MUSCLE

HIROYASU SATOH, KAZUNORI MATSUI, TOSHIAKI NAKATANI,
ITARU NARUSHIMA and TOSHIKATSU NAKASHIMA
Department of Pharmacology, Nara Medical University
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Summary: Electrophysiological effects of flecainide and ethacizin (class Ic antiarrhythmic drugs) were examined using conventional microelectrode techniques. Flecainide significantly depressed the maximum rate of depolarization ($\dot{V}_{max}$) at 3×10^{-6} M, and depolarized the resting potential (RP) at 10^{-5} M, in a concentration-dependent manner. Ethacizin depressed $\dot{V}_{max}$ at 10^{-6} M, and depolarized RP at 10^{-5} M, significantly. However, both drugs did not affect the effective refractory period (ERP) nor the action potential duration (75 % repolarization, APD_{75}). Both also had no effect on the action potential amplitude (APA). On the other hand, the drugs caused a use (or rate)-dependent block of $\dot{V}_{max}$, and their time constants of onset of inhibition (at 3 Hz) were slow; 6.3 ± 1.2 msec ($n=10$) in the presence of flecainide (10^{-5} M), and 6.0 ± 1.6 msec ($n=6$) in the presence of ethacizin (10^{-5} M). The time constants of the recovery were also so late: 12.2 ± 2.5 sec ($n=3$) for flecainide (10^{-5} M), and 27.1 ± 13.3 sec ($n=3$) for ethacizin (2×10^{-6} M). These results indicate that both antiarrhythmic drugs, flecainide and ethacizin, have no effect on APD_{75} and ERP, but possess the characteristics for very slow kinetics of the use-dependent block and the recovery for fast Na^+ channels of cardiac muscles. Ethacizin produces slower kinetics for the Na^+ channels than flecainide.

Index Terms

class Ic antiarrhythmic drugs, flecainide, ethacizin, slow kinetic, use-dependent block

INTRODUCTION

The mechanisms of the use-dependent block by the class I antiarrhythmic drugs for fast Na^+ channels of heart muscle has been investigated[1]. Class Ic antiarrhythmic drugs have no effect on the action potential duration (APD), and also possess characteristics for slow kinetics for a use-dependent block and recovery from the depression of fast Na^+ channels, according to a classification by Vaughan Williams[2] and Campbell[3]. Flecainide is well-known, and is classified as a class Ic antiarrhythmic drug, having characteristics of the slow kinetics[4)5].

Phenothiazines have been used in the treatment of a variety of psychiatric disorders[6]. The compounds depress the central nervous system, and their effects on autonomic nervous control include an antiadrenergic as well as an anticholinergic property. Phenothiazines may also depress the contractile performance of the heart, depress baroreceptor reflexes and exert antiarrhythmic effects similar to quinidine or lidocaine[6)7]. Ethmozin, a phenothiazine deriva-

tive, exhibits powerful antiarrhythmic properties[8)-12)]. A diethylamine analogue of ethmozin, ethacizin, has been recently developed as a new antiarrhythmic drug in Russia[13)14)] (Fig. 1). It has been demonstrated that ethacizin possesses a potent antiarrhythmic action[13)15)16)], and is a class I antiarrhythmic agent (an inihibitory action of fast Na^+ channels) in mammalian myocardium[17)].

In the present experiments, we sought to examine the electrophysiological effects of flecainide and ethacizin on the action potentials in canine ventricular muscle. Especially, the association time (a use-dependent block and a resting block) and the dissociation time (a recovery from blockade) of the drugs binding to the fast Na^+ channels were investigated.

MATERIALS AND METHODS

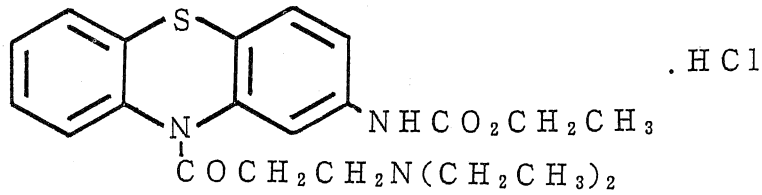
Preparations and recording

Eleven mongrel dogs of either sex, weighing 7-10 kg, were anesthetized with sodium pentobarbital (30 mg/kg, i. v.). The methods have been described in previous papers[18)-20)]. In brief, the heart was quickly excised, and the preparations (2-3 x 10-15 mm) were obtained from the right ventricle. The preparations were usually driven at 1 Hz. The duration of the stimuli was 1-2 msec and the voltage was about 50 % above the threshold. The action potential was obtained by a conventional glass microelectrode technique (its resistance was 5-10 MΩ), and recorded (displayed) on an oscilloscope (Nihon Kohden VC-11) and a thermal array recorder (Nihon Kohden, WS-641G), or photographed (Nihon Kohden RLG6201). The refractory period was measured at the 11th pulse with shorter intervals by interrupting the constant stimulation interval of 1 sec.

Analyses for the kinetics

Experiments for the use-dependent block of $\dot{V}_{max}$ were performed as represented in Fig. 2. The control value of $\dot{V}_{max}$ in the absence of drug was determined. Then, the drug was administrated and the stimulation was stopped. Following a resting period of 90 sec, repetitive stimulation was resumed at the same frequency as the control. For recovery of $\dot{V}_{max}$ inhibition, the stimulation at 3 Hz was stopped during exposure to drugs and the diastolic intervals were changed (Fig. 2). The percentage of recovery from use-dependent block was estimated by an equation: $1-(\dot{V}_{max})_{test}/(\dot{V}_{max})_{first}$, where $(\dot{V}_{max})_{test}$ is the value of $\dot{V}_{max}$ at the stimulation after

2-(Ethoxycarbonylamino)-10-[3-(diethylamino) propionyl]-phenothiazine hydrochloride



$C_{22}H_{27}N_3O_3S.\ HCl$; Mw : 449.99

Fig. 1. Chemical structure of ethacizin.

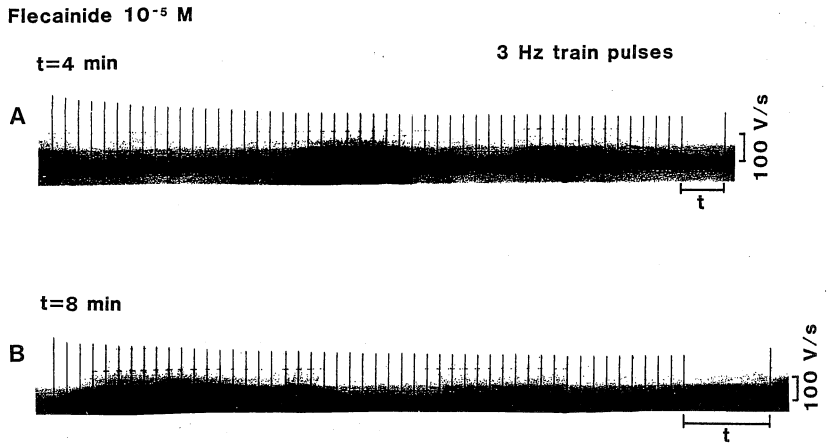


Fig. 2. Use-dependent inhibition of Na^+ channel and recovery from the depression at different intervals of pause. The use-dependent block the maximum rate of depolarization ($\dot{V}_{max}$) by flecainide 10^{-5}M is represented. The preparation was driven at 3 Hz. **A**: Pause for 4 min-duration. **B**: Pause for 8 min-duration. Note the difference of the $\dot{V}_{max}$ recovery from the depression in A and B.

the diastolic interval, and $(\dot{V}_{max})_{first}$ is that at the first stimulation after rest during exposure to the drug.

Values are given as mean±SD, and comparisons were Student's paired *t* test, as appropriate. Probability levels of less than 0.05 were taken as indicating significant differences.

Solutions

The composition of modified Tyrode solution (mM) was as follows: NaCl 137, KCl 4, $CaCl_2$ 1.8, $MgCl_2$ 1.1, $NaHCO_3$ 11.9, NaH_2PO_4 0.45, and glucose 5.5. The pH was adjusted to 7.4 with NaOH. The preparations were superfused in a bath with oxygenated (95 % O_2 and 5 % CO_2) Tyrode solution. The temperature was maintained at 36℃. The drugs used were flecainide acetate (Ricker Laboratories) and ethacizin, 2-(ethoxycarbonylamino)-10-[3-(diethylamino) propionyl]-phenothiazine hydrochloride, (Nikken Chemicals Ltd.). The drugs were administrated cumulatively.

RESULTS

Ethacizin (10^{-7} to 10^{-5} M) and flecainide (10^{-6} to 10^{-5} M) were cumulatively added to the bath solution. Both ethacizin (at over 10^{-7} M) and flecainide (at over $3x10^{-6}$ M) decreased $\dot{V}_{max}$ and depressed the amplitude (phase 0) of action potential, profoundly (Fig. 3A-B). The preparations were constantly stimulated at 1 Hz. The action potential duration at 75 % repolarization (APD_{75}) and the resting potential (RP) were unaffected by both drugs. The percentage changes in the action potential parameters are shown in Table 1. Flecainide depolarized RP and depressed the action potential amplitude at 10^{-5} M, and inhibited $\dot{V}_{max}$ at

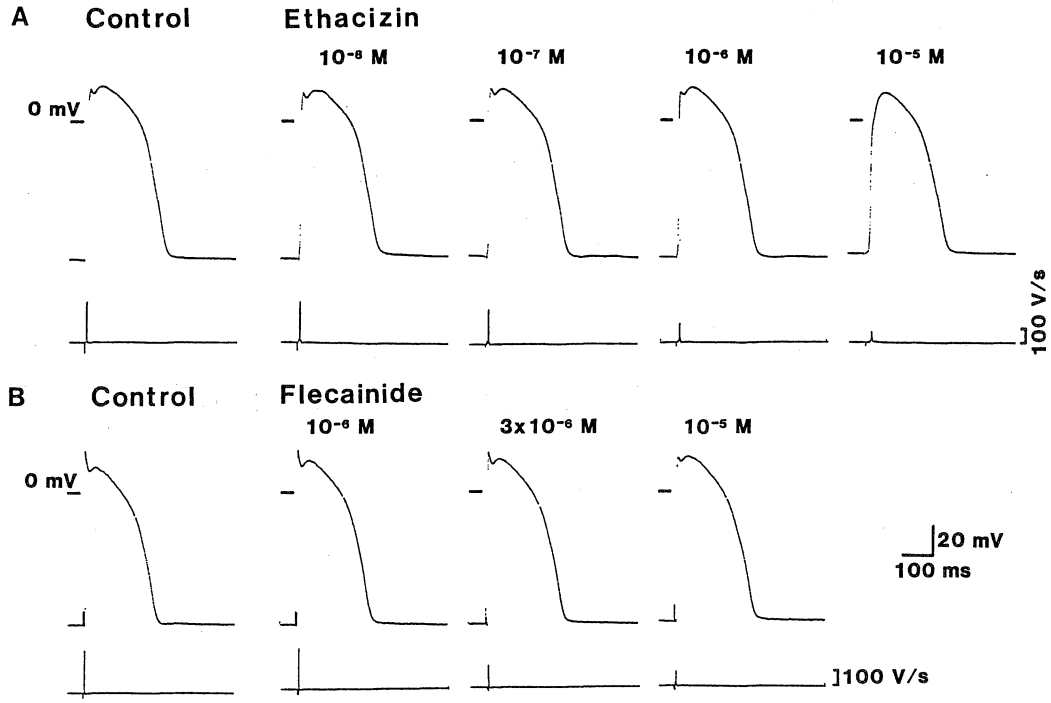


Fig. 3. Changes in the action potential configuration in the presence of ethacizin and flecainide. The preparatitns were stimulated at 1 Hz. **A**: Action potential and $\dot{V}_{max}$ in etacizin. **B**: Action potential and $\dot{V}_{max}$ in flecainide. Brief line at left of the action potential recordings is represented zero mV.

Table 1. Percentage changes in the action potential parameters in canine ventricular muscles in the presence of flecainide and ethacizin

	n	PR (mV)	APA (mV)	$\dot{V}_{max}$ (V/sec)	APD_{75} (msec)	ERP (msec)
Control	10	−87±6	101±14	257±10	218±28	214±21
Flecainide						
10^{-6}M	10	2±1%	1±2%	11±6%	1±1%	2±1%
$3x10^{-6}$M	10	4±3	1±1	12±5*	5±3	5±3
10^{-5}M	9	11±4*	8±3*	23±6**	6±3	9±4
Control	10	−88±5	112±10	254±21	220±24	216±32
Ethacizin						
10^{-7}M	10	1±1%	1±2%	6±2%	0%	0%
10^{-6}M	10	1±2	5±2	22±5**	4±2	1±2
10^{-5}M	7	6±2*	10±5	41±6**	4±2	1±3

Values represernt mean±SD. n: Number of experiments. RP: Resting Potential. APA: Amplitude of action potential. $\dot{V}_{max}$: Maximum rate of depolarization. APD_{75}: Duration of action potential at 75% repolarization. ERP: Effective refractory period. *: $P<0.05$, **: $P<0.01$, with respect to control values.

$3x10^{-6}$ M or more, significantly. Ethacizin depolarized RP at 10^{-5} M, and inhibited $\dot{V}_{max}$ at 10^{-6} M or more, significantly. Both drugs did not produce any effects on the APD_{75} and the effective refractory period (ERP), although both tended to prolong them.

After the stimulation was stopped, the drugs were added to the bath solution. At 90 sec after

the rest, the $\dot{V}_{max}$ of the action potential elicited by the first stimulation was decreased by about 10 % (concentration-dependent) as compared to control value (which is a resting block), and then the $\dot{V}_{max}$ declined during stimulation to a new steady state (which is a use- or frequency-dependent block), as shown in Figs. 4 and 5. The actual data in the presence of flecainide 10^{-5} M are also shown in Fig. 2A-B. The experiments were exerted at different frequencies of stimulations (1 to 3 Hz). The inhibition was frequency-dependent. The time constants (τ) of onset of inhibition of $\dot{V}_{max}$ at different stimulation frequencies in the presence of flecainide and ethacizin are summaried in Table 2. τ at 3 Hz was 6.3±1.2 msec (n=10) in flecainide 10^{-5} M and 6.0±1.6 msec (n=6) in ethacizin 10^{-5} M. These results indicate that ethacizin has a slow onset of inhibition for $\dot{V}_{max}$, like flecainide.

As shown in Fig. 2, the different intervals were exerted and the recovery of the $\dot{V}_{max}$ inhibition binding with Na^+ channels was examined. The preparations were driven by stimulation of 3 Hz. A typical example is shown in Fig. 6A-B. In the absence of drug, the recovery

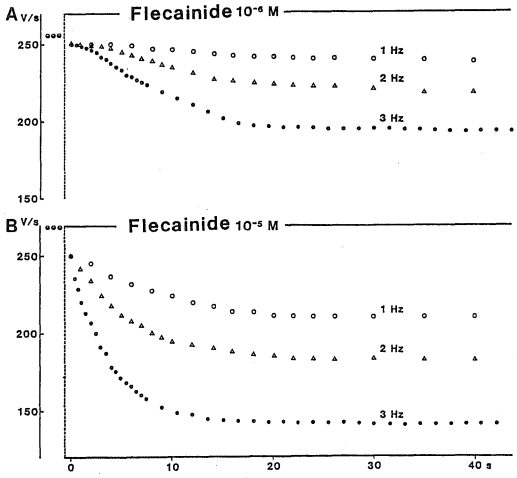


Fig. 4. Use-dependent block by ethacizin at different frequencies. **A**: Blockade in 10^{-7}M ethacizin. **B**: Blockade in 10^{-6}M ethacizin. Symbols are 1 Hz (open circles), 2Hz (triangles) and 3Hz (filled circles).

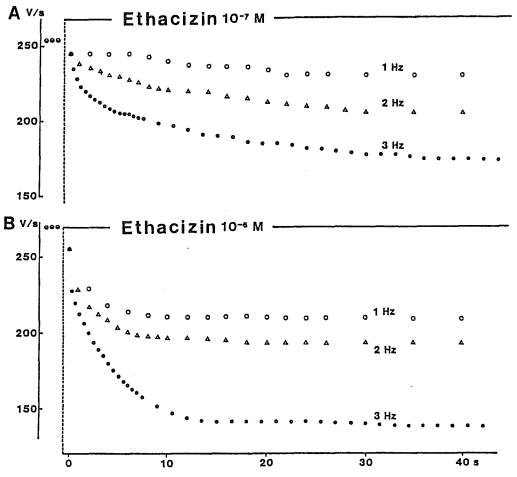


Fig. 5. Use-dependent block by flecainide at different frequencies. **A**: Blockade in 10^{-6}M flecainide. **B**: Blockade in 10^{-5}M flecainide. Symbols are 1Hz (open circles), 2Hz (triangles) and 3Hz (filled circles).

Table 2. Time constants of onset of inhibition for $\dot{V}_{max}$ at different frequencies of stimulation in the presence of flecainide and ethacizin

	n	1 Hz	2 Hz	3 Hz
Flecainide				
10^{-6}M	6	3.0±0.5 msec	3.3±0.6 msec	4.2±0.8 msec
3x10^{-6}M	5	3.6±0.8	3.6±0.7	4.2±1.1
10^{-5}M	10	4.5±1.2	5.4±1.7	6.3±1.2
Ethacizin				
10^{-6}M	10	3.3±0.8	3.3±0.5	3.6±0.7
2x10^{-6}M	6	3.3±0.7	3.6±0.9	4.2±0.6
10^{-5}M	6	4.5±1.4	6.1±1.9	6.0±1.6

Values represent the means±SD. n: Number of experiments.

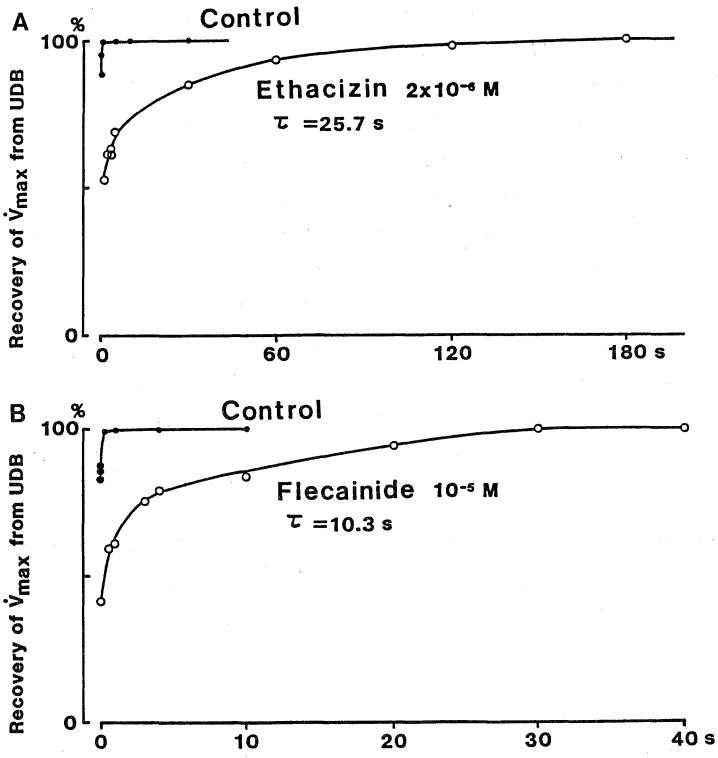


Fig. 6. Recovery of depressed Na^+ channel in the presence of ethacizin and flecainide. The data were obtained by constant stimulation at 3 Hz, except for the test of recovery from the depression of $\dot{V}_{max}$. The values (%) are represented along with time function. **A**: Ethacizin (2×10^{-6}M). τ was 25.7s. **B**: Flecainide (10^{-5}M). τ was 10.3s.

of $\dot{V}_{max}$ following the basic driven action potential was complete within 50 msec, and was well fitted by a single exponential function with a time constant of 22 ± 4 msec (n=8). In the presence of ethacizin, τ for the recovery was 26.8 ± 17.2 sec (n=4) at 10^{-6} and 27.1 ± 13.3 sec (n=3) at $2\text{x}10^{-6}$ M. On the other hand, in the presence of flecainide at 10^{-5} M, τ was 12.2 ± 2.5 sec (n=3). These results indicate that ethacizin possesses much slower kinetic for recovery from use-dependent block, twice that of flecainide.

DISCUSSION

It is now well established that many class I antiarrhythmic drugs (local anesthetic type) depress the $\dot{V}_{max}$ (or the fast Na^+ channels) of cardiac action potentials in a rate-dependent manner[2]. Their enhanced potencies at higher frequencies are thought to be brought about by selective binding of the drugs to inactivated Na^+ channels[21] or by voltage-dependent binding[22]. In a modulated receptor hypothesis[21)23)], rested Na^+ channels have a much lower affinity for antiarrhythmic drugs than depolarized (that is, activated and/or inactivated) channels. Furthermore, the more depolarized the holding potential is, the slower is the rate of recovery

from the block of the Na^+ channels.

Ethmozin is an effective, safe, and well-tolerated new antiarrhythmic drug[8)-12)]. A diethylamine analogue of ethmozin, ethacizin, had a negative inotropic effect in the presence of muscarinic and β-adrenoceptor blockades in ferret right ventricular papillary muscles[24)]. This is largely due to its recently demonstrated decreases of the slow inward Ca^{2+} current in frog atrial trabeculae[14)] and canine Purkinje fibers[8)14)24)]. Thus, ethacizin appear to be an even more potent antiarrhythmic drug[13)15)].

The present experiments show many similarities between flecainide and ethacizin. Neither flecainide nor ethacizin caused any significant effects on the APD and the ERP, although with both drugs, minor prolongations were produced. This is consistent with the results of Smetnev et al.[25)] and Rosenshtraukh et al.[13)]. These results indicate that ethacizin should be considered a class Ic antiarrhythmic drug, like flecainide.

On the other hand, ethacizin had almost the same kinetics as compared to those of flecainide : (1) slow kinetic for the onset of inhibition of $\dot{V}_{max}$, and (2) much slower kinetic for recovery from the depressed $\dot{V}_{max}$. Flecainide possesses well-known slow kinetics for the association and dissociation with Na^+ channels[4)5)26)]. The values in this study of the onset and recovery kinetics for flecainide are quite consistent with those reported previously. Therefore, we concluded that ethacizin produces the characteristics of slower kinetics for both the association and dissociation with fast Na^+ channels of cardiac muscles. Many factors to determine the rates of binding (association) and dissociation with the Na^+ channels are present. Drugs with high lipid solubility may produce a faster and greater block of the channels, whereas drugs with lower molecular weight may leave the channel more quickly during each diastolic interval of the action potentials[3)27)]. On the contrary, Hille[23)] showed that the rate of block development correlated well with lipid solubility, but not with molecular weight or size of the drug. The molecular weight of ethacizin is 449. 99. This is relatively large, indicating that ethacizin might have the slow kinetic characteristics for the channels. Courtney[28)] has shown that drugs having quite small dimensions (X, Y and Z) can gain access to the receptors during maintained depolarization (inactivated state), even if drugs had poor lipid distribution capabilities. For ethacizin, X=3 to 6 Å ; Y=6.5Å ; Z=15Å. The dimensions are quite different from the smaller dimensions of lidocaine which has fast kinetics for the Na^+ channels. Thus, the kinetics for association and dissociation might be dependent on the dimensions of drugs.

Abnormal excitability and cardiac arrhythmias result from the deformation of the normal matrix. Such an arrhythmogenic matrical configuration may be fixed or transient. To be effective antiarrhythmic drugs, drugs should normalize or further alter the matrix to prevent the arrhythmogenic matrical configuration. Clinically it has been found that ethacizin prolonged PR and QRS intervals, but had little or no effect on QT interval and refractory ventricular period. In addition, ethacizin had high antiarrhythmic efficacy in patients with ventricular premature beats[13)25)]. Therefore, these results in this study indicate that ethacizin as well as flecainide can produce antiarrhythmic matrical configurations directly as drugs with the slow kinetics, in a manner similar to other class I antiarrhythmic drugs, such as lidocaine and procainamide.

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