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An electrophysiological comparison of a novel class Ic antiarrhythmic agent, NIK-244 (ethacizin) and flecainide in canine ventricular muscle

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1 Electrophysiological effects of NIK-244 (ethacizin), a novel class I antiarrhythmic drug, were compared with flecainide in canine ventricular muscle by use of conventional microelectrode techniques.

2 At concentrations of 10^{-6} M or higher, NIK-244 depressed the maximum rate of rise of depolarization ($\dot{V}_{max}$) significantly in a concentration-dependent manner. Also, the resting potential was depolarized at 10^{-5} M. NIK-244 did not have any effect on the other action potential parameters or on the effective refractory period.

3 Flecainide significantly decreased $\dot{V}_{max}$ at 3×10^{-6} M or higher and the resting potential was depolarized at 10^{-5} M. Like NIK-244, flecainide did not affect other action potential parameters.

4 NIK-244 and flecainide caused a use-dependent block of $\dot{V}_{max}$, and the rates of onset of inhibition at 3 Hz stimulation were 0.014 ± 0.002 AP^{-1} at 2×10^{-6} M NIK-244 and 0.021 ± 0.012 AP^{-1} at 10^{-5} M flecainide. Under the same conditions, the time constants of the recovery from use-dependent block were 27.1 ± 13.3 s and 12.2 ± 2.5 s for NIK-244 and flecainide, respectively.

5 These results suggest that NIK-244, like flecainide, should be classified as a slow kinetic drug and as Ic.

Introduction

NIK-244 (ethacizin), 2-(ethoxycarbonylamino)-10-{3-(diethylamino)propionyl}-phenothiazine hydrochloride, has been developed in the U.S.S.R. and is a new antiarrhythmic drug of the phenothiazine group (Rosenshtraukh *et al.*, 1979; 1986). NIK-244 is the diethylamino analogue of ethmozin, which had been developed earlier as an antiarrhythmic agent (Rosenshtraukh *et al.*, 1979). It has recently been reported that NIK-244 possesses an even more powerful antiarrhythmic action than ethmozin (Shugushev *et al.*, 1984; Rosenshtraukh *et al.*, 1986). Recent results in mammalian myocardium (Nesterenko & Rosenshtraukh, 1983) suggest that NIK-244 is a novel class I antiarrhythmic agent according to the classification of Vaughan Williams (1980). Hageman *et al.* (1986) showed that NIK-244 neither reduced nor enhanced reflex-induced changes in sympathetic or parasympathetic actions. Clinically it has also been found that NIK-244 prolonged PR and QRS intervals but had little effect on QT interval and refractory ventricular period, and that NIK-244 had high antiarrhythmic efficacy in patients with ventricular premature beats (Smetnev *et al.*, 1983; Rosenshtraukh *et al.*, 1986).

The aim of the present experiments was to investigate the effects of NIK-244 on canine ventricular muscle. It was compared with flecainide, a class 1c and a slow kinetic antiarrhythmic drug (Campbell, 1983a), since it appears from the results of Rosenshtraukh *et al.* (1986) and Smetnev *et al.* (1983) that NIK-244 may not affect the action potential duration. Furthermore, we sought to determine the rate of onset of the inhibition and the recovery from use-dependent block of $\dot{V}_{max}$ in order to obtain more electrophysiological information.

Methods

Eight mongrel dogs of either sex, weighing 7–10 kg, were anaesthetized with sodium pentobarbitone (30 mg kg^{-1}, intravenously). The details of the

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Table 1 Changes in action potential parameters induced by NIK-244 in canine ventricular muscle

	n	*Resting potential* (mV)	*Action potential amplitude* (mV)	$\dot{V}_{max}$ (Vs^{-1})	*Action potential durations* (ms) 50%	90%	*Effective refractory period* (ms)
Control	11	−87 ± 4	111 ± 10	244 ± 27	169 ± 27	223 ± 25	220 ± 35
10^{-8} M	11	−87 ± 4	110 ± 10	237 ± 35	171 ± 25	220 ± 23	222 ± 31
10^{-7} M	11	−86 ± 3	110 ± 9	229 ± 51	172 ± 22	223 ± 22	221 ± 31
10^{-6} M	11	−86 ± 3	106 ± 6	190 ± 31**	168 ± 29	224 ± 19	223 ± 26
10^{-5} M	4	−82 ± 5*	97 ± 26	145 ± 19**	164 ± 28	231 ± 13	219 ± 32

The values represent mean ± s.d. *n*: number of experiments. * $P < 0.05$; ** $P < 0.01$, with respect to control values.

methods have been described in recent papers (Satoh & Hashimoto, 1986; Satoh *et al.*, 1987). In brief, the heart was quickly excised, and 3 to 5 preparations were obtained from the right ventricle of each dog. The preparations were driven at 1 Hz by a stimulator (Dia Medical System, DPS-160B). The duration of the stimuli was 1–2 ms and the voltage was about 50% above the threshold. The action potential was recorded by a conventional glass microelectrode technique (the electrode resistance was 5–10 MΩ) on an oscilloscope (Nihon Kohden VC-10) and a thermal array recorder (Nihon Kohden, WS-641G), and photographed (Nihon Kohden RLG-6201). The refractory period was measured at the 11th pulse with shorter intervals by interrupting the constant stimulation interval of 1 s.

To examine the use-dependent block of $\dot{V}_{max}$, experiments of the kind represented in Figure 1 were performed. In drug-free solution, the control value of $\dot{V}_{max}$ was determined. Then, NIK-244 was administered, and the stimulation was stopped. Following a resting period of 90 s, 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. 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 stimulation after the diastolic interval, and $(\dot{V}_{max})_{first}$ is that at the first stimulation after rest during exposure to the drug.

Perfusion solution

The composition of modified Tyrode solution (mM) was as follows: NaCl 137, KCl 4, $MgCl_2$ 2.7, $NaHCO_3$ 11.9, NaH_2PO_4 0.45 and dextrose 5.5. The pH was adjusted to 7.4 with NaOH. The preparations were superfused in a bath with oxygenated (97% O_2 plus 3% CO_2) Tyrode solution. The temperature was maintained at 36°C.

Drugs

The following drugs were used; NIK-244 (ethacizin HCl), which was kindly supplied by Nikken Chemicals Ltd., and flecainide acetate (Ricker Laboratories). These drugs were administered cumulatively.

Statistical analysis

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

Results

Effects of NIK-244 and flecainide on the action potential

At concentrations from 10^{-8} to 10^{-5} M, NIK-244 inhibited the maximum rate of depolarization ($\dot{V}_{max}$) in a concentration-dependent manner (Table 1). The other parameters of the action potential were unaffected. NIK-244 depressed $\dot{V}_{max}$ significantly at 10^{-6} M or higher. At a concentration of 10^{-5} M, the resting potential was depolarized. The action potential durations (APD) at 50% and 90% repolarizations were not lengthened and the effective refractory period was not increased to any significant extent.

Flecainide was also examined to compare its effects with those of NIK-244. Like NIK-244, flecainide inhibited $\dot{V}_{max}$ in a concentration-dependent manner, but did not cause any other effects on the action potential parameters. At 3×10^{-6} M or higher concentrations, flecainide depressed $\dot{V}_{max}$ significantly (Table 2). Flecainide, 10^{-5} M, depressed the action potential amplitude and depolarized the resting potential; it did not affect the action potential duration or the effective refractory period.

These results show many similarities between NIK-244 and flecainide and indicate that NIK-244 is a class Ic antiarrhythmic drug that produces no change in APD.

Table 2 Changes in action potential parameters induced by flecainide in canine ventricular muscle

	n	*Resting potential* (mV)	*Action potential amplitude* (mV)	$\dot{V}_{max}$ (Vs^{-1})	*Action potential durations* (ms) 50%	90%	*Effective refractory period* (ms)
Control	9	−82 ± 6	99 ± 14	260 ± 9	178 ± 30	216 ± 29	231 ± 10
10^{-6} M	9	−80 ± 7	98 ± 12	231 ± 88	172 ± 38	219 ± 37	235 ± 13
3×10^{-6} M	9	−79 ± 9	98 ± 12	228 ± 33*	182 ± 34	226 ± 33	242 ± 14
10^{-5} M	8	−73 ± 11*	91 ± 15*	200 ± 41**	187 ± 30	228 ± 29	253 ± 16

The values represent means ± s.d. *n*: number of experiments. * $P < 0.05$, ** $P < 0.01$, with respect to control values.

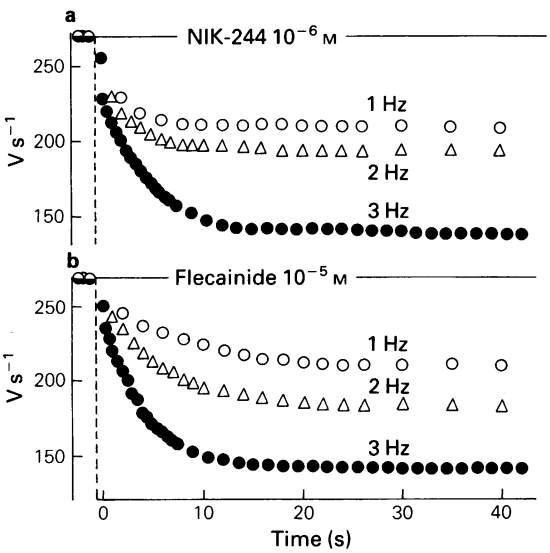

Figure 1 Time courses of inhibition of $\dot{V}_{max}$ at different stimulation frequencies in the presence of NIK-244 (a) and flecainide (b). Both drugs depressed $\dot{V}_{max}$ in a use-dependent manner. The ordinate scale shows magnitude of $\dot{V}_{max}$, and the abscissa scale represents before and during drug administration at times (0–40 s) after the onset of stimulation.

Use-dependent block of $\dot{V}_{max}$

As shown in Figure 1, the $\dot{V}_{max}$ at the first action potential was decreased (a resting block), and declined during stimulation to a new steady-state (a use- or a frequency-dependent block). NIK-244 inhibited $\dot{V}_{max}$ frequency-dependently. The decline curve at 1 Hz was unaffected at 10^{-7} M, but in the presence of 10^{-6} M NIK-244 it became steeper (Figure 1a). After the rest, $\dot{V}_{max}$ of the first action potential was 258 $V s^{-1}$. The resting block was 4.4% ($\dot{V}_{max}$ was 270 $V s^{-1}$ in drug-free solution). At 1 Hz $\dot{V}_{max}$ was 213 $V s^{-1}$; at 2 Hz, 193 $V s^{-1}$; and at 3 Hz, 141 $V s^{-1}$ as a new steady-state.

Application of 10^{-5} M flecainide also caused a marked use-dependent block (Figure 1b). Flecainide depressed $\dot{V}_{max}$ from 269 $V s^{-1}$ in drug-free solution to 235 $V s^{-1}$ at 1 Hz, to 183 $V s^{-1}$ at 2 Hz and to 139 $V s^{-1}$ at 3 Hz. The resting block was 7.1%.

These findings indicate that NIK-244, like flecainide, is an antiarrhythmic drug with slow rate of onset of $\dot{V}_{max}$ inhibition.

Rate of onset of $\dot{V}_{max}$ *inhibition*

Campbell (1983a, b) showed that antiarrhythmic drugs can be classified by the rate of onset of blockade in the presence of concentrations which caused

Table 3 The rate of onset of inhibition of the maximum rate of depolarization at different frequencies of stimulation by NIK-244 and flecainide

	n	1	2	3 Hz
NIK-244				
10^{-6} M	11	0.011 ± 0.028	0.011 ± 0.026	0.012 ± 0.009
2×10^{-6} M	3	0.011 ± 0.018	0.012 ± 0.022	0.014 ± 0.002
10^{-5} M	3	0.015 ± 0.016	0.021 ± 0.011	0.020 ± 0.019
Flecainide				
10^{-6} M	5	0.010 ± 0.024	0.011 ± 0.021	0.014 ± 0.026
3×10^{-6} M	4	0.012 ± 0.014	0.012 ± 0.011	0.014 ± 0.014
10^{-5} M	13	0.015 ± 0.009	0.018 ± 0.003	0.021 ± 0.012

Values represent the means ± s.d. *n*: number of experiments.
The unit of values is AP^{-1}.

about 50% of the use-dependent depression of $\dot{V}_{max}$ at an interstimulus interval of 300 ms (approximately equivalent to 3 Hz). Thus, in the present experiments, using concentrations of 10^{-6} M (47.8% inhibition) and 2×10^{-6} M (56.3% inhibition) of NIK-244, the rates of onset of $\dot{V}_{max}$ inhibition were $0.012 \pm 0.009\,AP^{-1}$ ($n = 11$) and $0.014 \pm 0.002\,AP^{-1}$ ($n = 3$), respectively. When stimulation started, the upstroke velocities declined in an exponential fashion, action potential by action potential, as shown in Figure 1. The logarithms of the decremental losses of $\dot{V}_{max}$ of the action potentials during the train were plotted against pulse number. Thus, the unit of AP^{-1} represents the reciprocal of the number of action potentials which corresponds to the time constant for the decay of $\dot{V}_{max}$. The results obtained at different frequencies and concentrations are summarized in Table 3.

On the other hand, the $\dot{V}_{max}$ was inhibited by 37% and 58% in the presence of flecainide 3×10^{-6} M and 10^{-5} M, respectively. At 3 Hz, the rates of onset of $\dot{V}_{max}$ inhibition were $0.014 \pm 0.014\,AP^{-1}$ ($n = 4$) in 3×10^{-6} M and $0.021 \pm 0.012\,AP^{-1}$ ($n = 13$) in 10^{-5} M flecainide. The rates of onset of $\dot{V}_{max}$ inhibition were examined at different frequencies and in the presence of various concentrations (Table 3).

These results indicate that NIK-244 had slow kinetics of $\dot{V}_{max}$ inhibition, like flecainide.

Recovery of $\dot{V}_{max}$ from use-dependent block

Figure 2 illustrates a typical example, in which $\dot{V}_{max}$ of the premature action potential is plotted against diastolic interval (abscissa scale). In the absence of drug, the recovery of $\dot{V}_{max}$ following the basic driven action potential was complete within 50 ms and was well fitted by a monoexponential function with a time constant (τ) of 22 ± 4 ms ($n = 8$). In the presence of 10^{-6} M and 2×10^{-6} M NIK-244, the time constants for the recovery were 26.8 ± 17.2 s ($n = 4$) and 27.1 ± 13.3 s ($n = 3$), respectively. In contrast, at 10^{-5} M flecainide, it was 12.2 ± 2.5 s ($n = 3$). Note that the time constant for NIK-244 was much slower, almost twice that of flecainide.

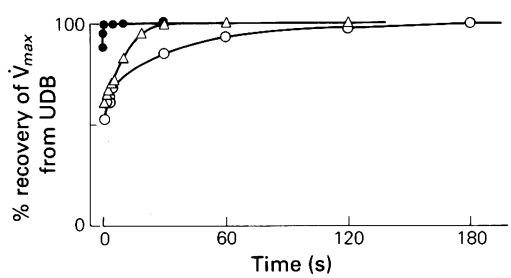

Figure 2 Recovery of $\dot{V}_{max}$ from use-dependent block (UDB). The percentage recovery was plotted against the diastolic interval. (●) Control, the time constant (τ) was 25 ms in drug-free solution; (△) flecainide 10^{-5} M, $\tau = 10.3$ s; (○) NIK-244 2×10^{-6} M, $\tau = 25.7$ s.

These results show that NIK-244 is an antiarrhythmic agent with a much slower recovery from use-dependent block than flecainide.

Discussion

Class I antiarrhythmic drugs (local anaesthetic type) are effective in the treatment of certain kinds of cardiac arrhythmias. These drugs depress the fast Na channel responsible for action potential generation. Hondeghem & Katzung (1977) and Hille (1977) in recent reviews have emphasized that the rapid upstroke phase of cardiac action potentials is blocked in a use-dependent manner during exposure to an antiarrhythmic drug (modulated receptor hypothesis). Rested sodium channels have a much lower affinity for antiarrhythmic drugs than depolarized (activated or inactivated) channels. In addition, the more depolarized the holding potential is, the slower is the rate of recovery from block.

Usually it is considered that the classifications of Class I antiarrhythmic drugs by effects on APD and by the rate of onset of $\dot{V}_{max}$ inhibition and recovery from use-dependent block are similar, but both were quite different. The former is divided into further three subclasses; antiarrhythmic drugs causing the prolongation of APD are in subclass Ia, those causing the shortening of APD are in Ib, and drugs with no change in APD are in Ic. On the other hand, there are marked differences among drugs in the speed at which $\dot{V}_{max}$ falls to a new plateau level and then recovers to an initial level during exposure to an antiarrhythmic drug. Therefore, class I antiarrhythmic drugs have been divided into three distinct subclasses by differences on their interaction with fast Na^+ channel (binding and dissociation kinetics); i.e., fast, intermediate and slow kinetic drugs (Harrison *et al.*, 1981; Campbell, 1983a).

Action potential duration

In the present experiments, neither NIK-244 nor flecainide caused any significant effects on APD or on the effective refractory period, although with both drugs minor prolongations were observed. This is consistent with the results of Smetnev *et al.* (1983) and Rosenshtraukh *et al.* (1986), who have already shown that intravenous NIK-244 causes no significant change in ventricular refractory periods and QT interval. Like flecainide, therefore, NIK-244 should be considered a class Ic antiarrhythmic drug.

NIK-244 decreases the slow inward current as well as the fast inward current in frog atrial trabeculae

and canine Purkinje fibres (Rosenshtraukh *et al.*, 1979; Chazov *et al.*, 1984; Urthaler *et al.*, 1986). Since NIK-244 had no effect on APD it may also decrease time-dependent outward current. Voltage-clamp experiments are required to ascertain this possible mechanism of action.

Block development and recovery of $\dot{V}_{max}$

In the present experiments, NIK-244 did not cause any effect on APD but inhibited $\dot{V}_{max}$, like flecainide. Comparing NIK-244 and flecainide, the rate of onset of use-dependent block was similar, being $0.014\,AP^{-1}$ and $0.021\,AP^{-1}$, respectively. The time constant of recovery for NIK-244 was approximately twice that of flecainide. These values of the rate of onset of $\dot{V}_{max}$ inhibition and the recovery for flecainide are consistent with those reported previously (Campbell, 1983a, b), although animal species are different. According to Harrison *et al.* (1981) and Campbell (1983b), the values for the fast kinetic drugs are more than $0.277\,AP^{-1}$, those for the intermediate ones in the range from 0.113 to $0.055\,AP^{-1}$, and those for the slow ones less than $0.029\,AP^{-1}$. In the present experiments, the rates of onset of $\dot{V}_{max}$ inhibition for both NIK-244 and flecainide were less than $0.029\,AP^{-1}$. We, therefore, conclude that NIK-244 should be classified as an antiarrhythmic drug with slow kinetics of action. This is confirmed by the fact that NIK-244 slowed the recovery time from use-dependent block to twice that of flecainide.

There are many factors that determine the binding or the dissociation rates for the fast Na channel. Courtney (1980) and Campbell (1983b) showed that drugs with higher lipid solubility may produce a faster and greater block, whereas drugs with lower molecular weight may leave the channel more quickly during each diastolic interval of the action potentials. On the other hand, Hille (1977) reported 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 NIK-244 is 449.99, and is relatively large, indicating that NIK-244 might have slow kinetic characteristics. More recently, Courtney (1988) has shown that drugs having quite small dimensions (X, Y and Z) can gain access to the receptors during maintained depolarization (inactivated state), despite poor lipid distribution capabilities. In NIK-244, X = 3–6; Y = 6.5; Z = 15 Å, which are quite different from the smaller dimensions of lidocaine with fast kinetics. Extensive studies are required to elucidate the mechanism of the kinetics more clearly.

In conclusion, the present results show many similarities between NIK-244 and flecainide, such as no change in APD and effective refractory period, the slow rate of onset of $\dot{V}_{max}$ inhibition, and the slow kinetics of recovery from use-dependent block. It is, therefore, suggested that the new antiarrhythmic agent, NIK-244 (ethacizin), like flecainide, has slow kinetics of action.

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