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Chronotropic effect of nizofenone fumarate in rabbit sino-atrial node *in vitro*

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1 The effects of nizofenone fumarate were studied on the membrane potentials and currents of rabbit sino-atrial node preparations by means of the double-microelectrode voltage clamp method.

2 In spontaneously firing pacemaker cells, nizofenone (above 1 μM) decreased the heart rate. Above 3 μM, nizofenone reduced the maximum upstroke velocity, the amplitude of the action potential and the slope of the phase 4 depolarization, and prolonged the action potential duration at 50% repolarization.

3 Under voltage clamp conditions, nizofenone decreased the slow inward current and the time-dependent potassium outward current in a dose-dependent manner.

4 These findings suggest that nizofenone exerts an inhibitory action on the automaticity of sino-atrial node preparations via effects on both inward and outward currents.

Introduction

Nizofenone fumarate has been demonstrated to exert a protective effect against experimentally-induced cerebral ischaemia and anoxia (Yasuda *et al.*, 1978; 1979; Tamura *et al.*, 1979). In anaesthetized dogs, nizofenone also induces negative chronotropic and inotropic actions, and prolongs PQ and QT intervals of the electrocardiogram (Nakamura *et al.*, 1984). Furthermore, animal experiments have shown that nizofenone possesses an antiarrhythmic property and its mechanism can be explained by a so-called 'quinidine-like' action (Davis & Temte, 1968; Miura *et al.*, 1985). However, the electrophysiological effects of nizofenone on cardiac tissues have not been fully clarified.

In the present study, we investigated the effects of nizofenone on the action potentials and the membrane currents of rabbit sino-atrial node cells, in order to clarify the ionic mechanism underlying its negative chronotropic effect in this pacemaker tissue.

Methods

Rabbits of either sex (1.5 to 2.0 kg) were killed by a blow to the neck and their hearts immediately isolated. After removing the right atrium, strands of sino-atrial tissue of 1 mm in length and 0.3 mm in width were prepared by dissecting the tissue in the direction perpendicular to the crista terminalis. These strands were placed in a recording chamber (0.5 ml volume) and were ligated at two sites with a fine silk fibre to give a final dimension of about 0.3 × 0.3 mm. The method of preparing the small sino-atrial node specimens has been described in detail previously (Noma & Irisawa, 1976).

The composition of Tyrode solution was (in mM): NaCl 137.0, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.0, NaH_2PO_4 0.6 and its pH was adjusted to 7.4 by adding Na_2HPO_4. The temperature of the perfusates and the recording chamber was maintained at 36–37°C throughout the experiments.

In the voltage clamp experiments, two microelectrodes filled with 3 M KCl (10–30 MΩ) were used, one was to record the membrane potential and the other to apply the current intracellularly. The membrane potential and current were displayed on an oscilloscope (Kikusui Denshi, 5516 ST) and recorded with a pen-recticorder (Nihon Kohden, RJG-4100). The principle of the feedback circuit was the same as that described by Noma *et al.* (1980), and essentially the same ground amplifier as used by New & Trautwein (1972) was employed. When both microelectrodes recorded identical action potentials, the preparation was clamped to −40 mV (holding potential), where the holding current is nearly zero (Noma *et al.*, 1979).

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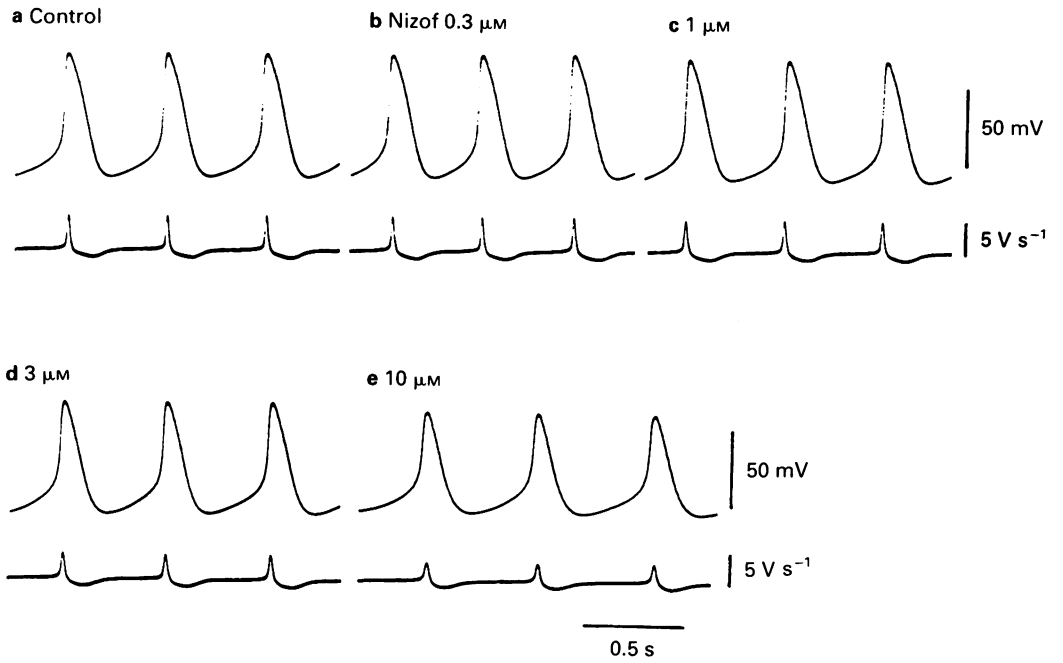

Figure 1 Effects of increasing concentrations of nizofenone (Nizof) on transmembrane action potentials of rabbit sino-atrial node preparations. Upper trace shows the action potential and lower trace shows its first derivative.

Electrophysiological measurements were made 15 min after changing to a new solution. In voltage clamp experiments the amplitude of the slow inward current was measured as the difference between the peak inward current and the current 100 ms after the onset of the clamp pulse (McDonald & Trautwein, 1978; Satoh & Hashimoto, 1986).

For the various characteristics of the action potentials and membrane currents, the values are presented as mean ± s.d. Statistical analysis was performed by use of Student's t test, and P values less than 0.05 were considered significant.

Nizofenone fumarate was kindly supplied from Yoshitomi Pharmaceutical Industries Ltd.

Results

Effects of nizofenone on membrane potentials

The effects of nizofenone on action potentials of rabbit sino-atrial node pacemaker cells were examined by means of the conventional glass microelectrode method. Figure 1 shows an example of the

Table 1 Effects of nizofenone on various parameters of sino-atrial node action potentials of rabbits

	SCL (ms)	$\dot{V}_{max}$ ($V s^{-1}$)	*RDD* ($mV s^{-1}$)	*APA* (mV)	APD_{50} (ms)
Control	404.0 ± 60.2	7.5 ± 2.4	90.4 ± 17.4	91.5 ± 8.8	98.5 ± 13.0
Nizofenone					
0.3 μM	404.5 ± 47.4	7.3 ± 1.9	90.4 ± 17.4	91.9 ± 8.8	99.9 ± 12.7
1 μM	431.2 ± 54.0*	7.0 ± 2.5	86.1 ± 19.9	91.1 ± 9.5	101.3 ± 13.8
3 μM	493.1 ± 58.6***	5.6 ± 2.2**	67.5 ± 15.6**	87.0 ± 10.9*	110.3 ± 12.7**
10 μM	626.3 ± 111.0***	3.1 ± 1.9***	51.6 ± 10.9***	70.1 ± 11.9***	112.1 ± 9.7***

SCL: spontaneous cycle length, $\dot{V}_{max}$: maximum rate of rise, RDD: rate of diastolic depolarization, APA: action potential amplitude, APD_{50}: action potential duration at 50% repolarization. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ with respect to control values.

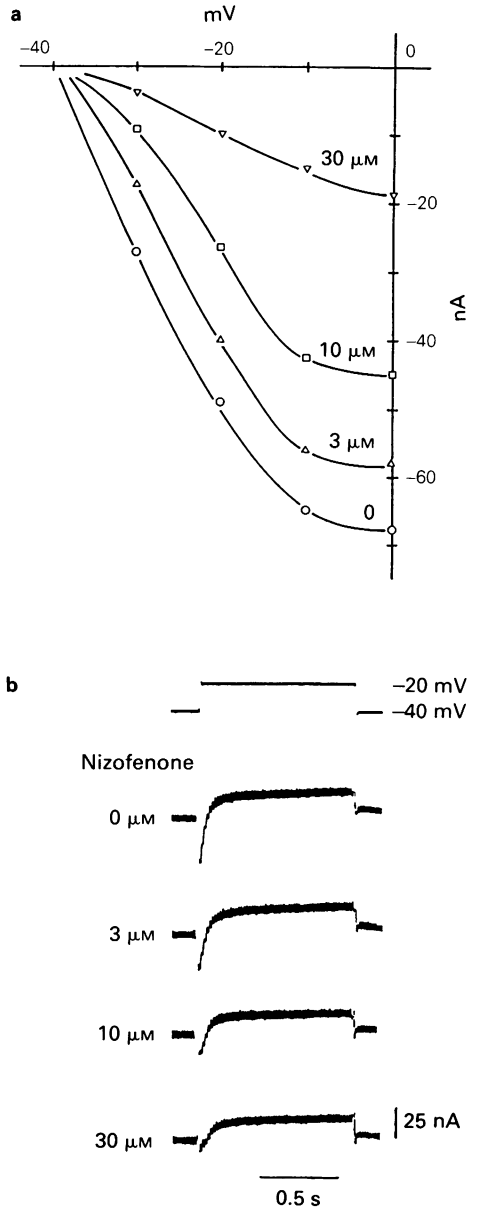

Figure 2 Effects of nizofenone on the slow inward current (I_{si}) of rabbit sino-atrial node. (b) Shows the voltage clamp traces in the absence and presence of nizofenone. (a) Shows the current-voltage relationship for I_{si} before (○) and after exposure to 3 μM (△), 10 μM (□) and 30 μM (▽) nizofenone.

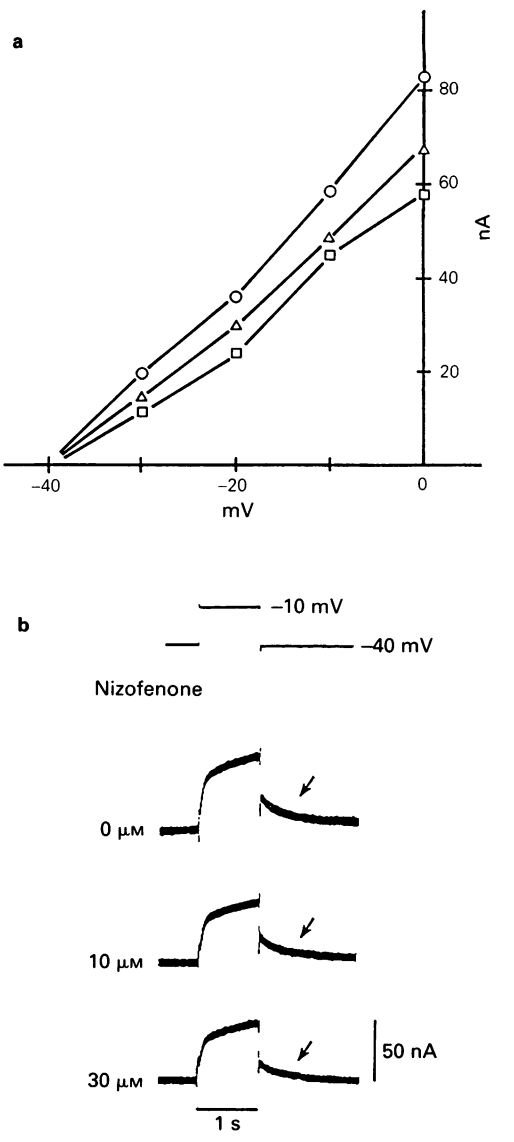

Figure 3 Effects of nizofenone on the potassium outward current (I_K). (b) Shows the voltage clamp traces in the absence and presence of nizofenone, where the cells had been pretreated with verapamil before the experiment. Arrows indicate the tail currents after repolarization. (a) Depicts the current-voltage relationship for the current amplitude measured at 1 s after the test pulse before (○) and after exposure to 10 μM (△) and 30 μM (□) nizofenone.

effects of cumulative concentrations of nizofenone. Although 0.3 μM nizofenone produced no significant changes in the membrane potentials, several effects became evident at concentrations above 1 μM. Above 3 μM, nizofenone increased the spontaneous cycle length (SCL), and decreased the maximum rate of

depolarization ($\dot{V}_{max}$), the action potential amplitude (APA) and the rate of the diastolic depolarization (RDD) in a dose-dependent manner. The action potential duration at 50% repolarization (APD_{50}) was also prolonged significantly at above 3 μM. With a high concentration (30 μM), nizofenone inhibited the spontaneous discharge completely in 9 out of 10 preparations. The effects of nizofenone on various electrophysiological characteristics of sino-atrial node action potentials obtained in 10 preparations are summarized in Table 1.

Effects of nizofenone on membrane currents

In order to clarify the ionic mechanism underlying the effect of nizofenone, voltage clamp experiments were performed. Figure 2 shows the effect of increasing concentrations of nizofenone on the slow inward current (I_{si}). A depolarizing step from the holding potential of -40 mV elicited I_{si} followed by a gradually increasing outward current (Figure 2b). Figure 2a shows the current-voltage relationship for I_{si} before and after exposure to nizofenone. As can be seen (Figure 2a) nizofenone obviously reduced the I_{si} amplitude in a dose-dependent manner. In four experiments of this kind, the I_{si} amplitude at -10 mV was reduced by a factor of 0.51 ± 0.10 ($P < 0.01$) after exposure to 10 μM nizofenone.

In order to investigate the effect of nizofenone on the outward current, a calcium antagonist (verapamil) was used. As shown in Figure 3, in the presence of verapamil (2 $\mu g\,ml^{-1}$), I_{si} was almost completely blocked. Therefore, the slowly developing outward current during a depolarizing test pulse reflects the activation of I_K, whereas the tail current after repolarization to the holding potential shows its deactivation. The current-voltage relationship for the current measured at 1 s after the test clamp is shown in Figure 3a, suggesting that nizofenone decreased the steady-state current amplitude during depolarization. In three experiments, nizofenone (10 μM) decreased the steady-state current amplitude at -10 mV by a factor of 0.74 ± 0.06 ($P < 0.01$). In Figure 4a, the tail current amplitude was plotted against the membrane potential. These observations show that nizofenone reduced not only the steady-state current amplitude during depolarization but also the tail current amplitude after repolarization, indicating a reduction of the time-dependent potassium outward current (I_K). In Figure 4b, the series of the tail currents were normalized by plotting the amount of tail current at 0 mV as 1.0 against the membrane potentials of test pulses. Since these curves obtained with and without nizofenone are almost superimposable, it is unlikely that nizofenone alters the degree of activation of I_K as a function of the membrane potential (Noma *et al.*, 1980; Senami & Irisawa, 1981; Kotake *et al.*, 1987). Similar results were obtained in three preparations.

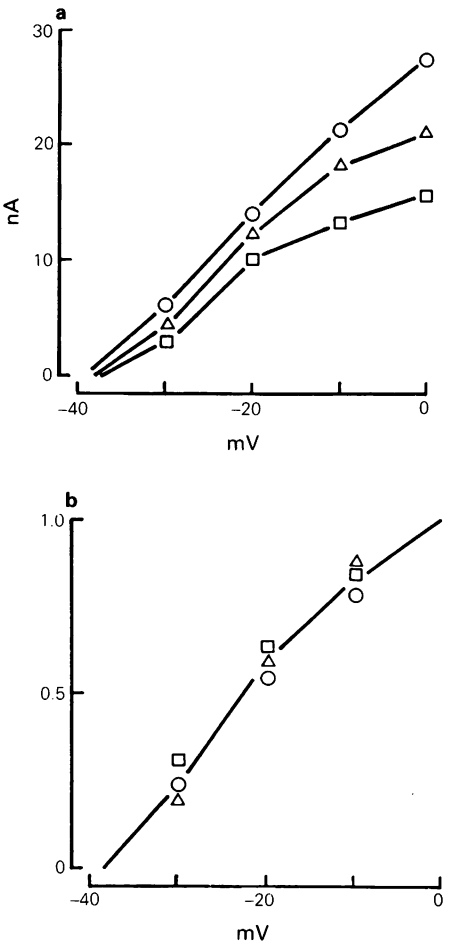

Figure 4 Effects of nizofenone on the tail current and the steady-state activation of the potassium outward current (I_K). (a) Relationship between the peak outward tail current amplitude and the membrane potential in the absence (○) and presence of 10 μM (△) and 30 μM (□) nizofenone. (b) Relative outward current tail normalized with respect to the current recorded at 0 mV (ordinate scale) as a function of the membrane potential (abscissa scale) (symbols as in (a)). Values were obtained from the same preparation as that in Figure 3.

Discussion

Nizofenone, an agent which has been shown to exert a protective effect against experimental cerebral ischaemia and anoxia, caused a significant prolongation of spontaneous cycle length (SCL) at concentrations

above 1 μM. Above 3 μM, the compound also decreased the maximum upstroke velocity ($\dot{V}_{max}$), the action potential amplitude (APA) and the rate of the diastolic depolarization (RDD), whereas the action potential duration at half-amplitude (APD_{50}) was prolonged. Since $\dot{V}_{max}$ of sino-atrial node cells is almost entirely dependent on I_{si} (Irisawa, 1978) and since I_{si} plays an important role in the development of diastolic depolarization (Noma *et al.*, 1980), these results suggest that nizofenone reduces Ca^{2+} influx through the cell membrane. In the present study, using voltage clamp conditions we demonstrated that nizofenone depresses I_{si} and I_K in a dose-dependent manner.

The nizofenone-induced bradycardia was accompanied by a deceleration of the phase 4 depolarization. Based on the voltage clamp experiments, the diastolic depolarization of sino-atrial pacemaker cells occurs as a result of the combined effects of deactivating I_K and activating I_{si} and the hyperpolarization activated current (I_h; Noma *et al.*, 1980; Brown, 1982; Irisawa & Noma, 1984). However, there is evidence that I_h does not have a significant contribution to the net membrane current. The threshold of activation of I_h is approximately -60 mV and its time constant at -70 mV is in the order of seconds in sino-atrial node cells (Noma *et al.*, 1980). A chronotropic effect can be seen in the cell having a diastolic potential at around -60 mV. Furthermore, Noma *et al.* (1983) showed that a positive chronotropic effect of adrenaline can be demonstrated even in cells where I_h is almost completely blocked by Cs. Therefore, it seems likely that a reduction of I_{si} induced by nizofenone would decelerate the phase 4 depolarization, resulting in a negative chronotropic effect. Furthermore, nizofenone prolonged APD_{50}. The prolongation of APD is generally explained by a decrease in the outward current and/or an increase in I_{si}. The present study favours a decrease in I_K as the main genesis of APD prolongation and this might also contribute to the bradycardiac action.

Previous *in vitro* experiments (Miura *et al.*, 1985) showed that nizofenone decreased $\dot{V}_{max}$ and prolonged APD in guinea-pig papillary muscle and dog Purkinje fibres, implying that the agent has a quinidine-like action on the cardiac membrane. Nizofenone also suppressed the spontaneous activity of dog Purkinje fibres and guinea-pig sino-atrial node, and decreased the contractile force of guinea-pig papillary muscle (Miura *et al.*, 1985). These observations suggest that nizofenone inhibits both the fast Na^+ and the slow Ca^{2+} inward currents. In this study, we confirmed that nizofenone depressed the sino-atrial node pacemaker activity by decreasing I_{si} and I_K under voltage clamp conditions. We have also shown that several class I antiarrhythmic agents such as aprindine, cibenzoline, disopyramide and mexiletine suppress I_{si} and I_K of rabbit sino-atrial node cells (Kotake *et al.*, 1985; 1986; 1987; Igawa *et al.*, 1986), which is consistent with the membrane current changes induced by nizofenone.

In conclusion, these electrophysiological observations suggest that nizofenone exerts inhibitory actions on the electrical activity of both fast- and slow-response fibres of the heart. In sino-atrial node preparations, nizofenone does not have an effect on a single current system, but it depresses the automaticity of pacemaker cells due to a reduction of I_{si} and I_K.

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