BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1994 Jan; 15 (1): 28—32

Frequency- and voltage-dependent inhibition of delayed outward potassium current by flecainide in isolated atrial cell of guinea pig heart

YANG Bao-Feng, DU Zhi-Min[1], KOHEI Sawada[2]
(Department of Pharmacology and [1]Clinical and Pharmaceutical Institute, Harbin Medical University, Harbin 150086, China; [2]Department of Pharmacology, Eisai Tsukuba Research Laboratories, 1—3, 5-Chome, Ibaraki 300—26, Japan)

ABSTRACT Effects of flecanide (Fle) on membrane currents were studied using an isolated single atrial cell from guinea pig hearts. The tight-seal cell clamp technique was used. In the current clamp condition, Fle prolonged singnificantly the atrial action potential (APD) with frequency dependence.

Delayed ourward K^+ current and outward tail current w re specifically inhibited by Fle in a frequency- and concentration-dependent fashion. Fle inhibited I_k more strongly as the membrane potential became more positive from +10 mV to +60 mV. The value of I_k was attenuated to 973 pA from 1105 pA of control and the value of tail current was reduced to 113 pA from 288 pA of control at 60 mV. The drug did not affect the holding current.

The effects of Fle on the action potential and transmembrane ionic currents strongly suggested that the main mechanism of action of this agent was to inhibit the voltage-dependent potassium current. In the voltage clamp condition, Fle affected neither the conventional L type Ca^{2+} current nor the I_{ki} current significantly. Our research proved that Fle was not completely consistent with the class Ic agents, because Fle could markedly increase the APD in the experiment.

KEY WORDS flecainide; heart atrium; electrophysiology

Flecainide (Fle) has been reported to be effective in suppressing the atrial[1] as well as ventricular arrhythmias in animals and clinical studies[2,3]. The mechanism of Fle for the suppression of ventricular arrhythmias was thought to be mainly due to depression of Na-channels[4]. However, the precise electrophysiological mechanism involved in the prevention of atrial arrhythmias remains not yet fully understood. Thus, we examined the ionic mechanism underlying the prolongation of action potential by Fle, using the suction pipette and whole cell patch clamp techniques.

Received 1992-07-07 Accepted 1993-11-16

MATERIALS AND METHODS

Isolation of single atrial cells Isolation of single cells was done using an enzymatic dissociation procedure similar to that described by Kurachi *et al*[5]. Briefly, guinea pig of either sex weighing 321±*s* 63 g were anesthetized with pentobarbital sodium (40 to 50 $mg \cdot kg^{-1}$). After the heart was dissected out and the blood was washed away, 60 ml of Ca^{2+} free Tyrode solution was perfused through the coronary arteries with a Langendorff apparatus. Then, 50 ml of low Ca^{2+} Tyrode solution containing collagenase 0.4 $mg \cdot ml^{-1}$ (Sigma, type 1) and $CaCl_2$ 50 $\mu mol \cdot L^{-1}$ instead of 1.8 $mmol \cdot L^{-1}$ was recirculated through the heart about 30 min. Thereafter, the collagenase was washed away by 100 ml of KB solution.

The isolated cells were then put into a recording chamber. The temperature of superfusates in the recording chamber was kept at 35 ℃. The composition of the Tyrode solution was similar to that described by Hiraoka M, *et al*[6].

Recordings of membrane potentials and membrane currents To record the membrane potential and current, the patch clamp amplifier (EPC-7, List Co., Germany) was used. After a tight sealing between the electrode tip and the cell membrane was established and the cell membrane at the tip of the electrode was ruptured, resting membrane potential of about −80 mV was usually recorded. Current and voltage signals were stored on a magnetic tape of the data-recorder (A-45, Sony Co.) for a later analysis. Signals were also inscribed using a recorder (Omnicorder 8 M 14, NEC San-ei Japan), which had a linear frequency response up to 2.5 kHz. The stability of the prepara-

tions was judged by observing the action potential duration at 90 % repolarization or the amplitude of the peak calcium current. If either parameter did not vary, more than 5 % of their values during 5 min of observation period, the cells were assumed to be in a stable condition and further experiment was proceeded. Our preparations usually responded to electrical stimulation or voltage clamp pulses in a stable fashion for 50 — 70 min. Thus, our experiments were completed within 45 min.

Data analysis The membrane currents were monitored by an oscilloscope (COS 5020 ST Kikusui, Tokyo) and were stored in a video casette recorder (BR 6400, Victor, Tokyo) using PCM converter system (RP-880). The data were reproduced and analyzed with a computer (NEC, Tokyo). The paired t test was used for statistical analysis of the data. ($P<0.05$ was considered significant).

RESULTS

Prolongation of action potential The action potential of an atrial cell was evoked with a constant current (5 ms in duration) passing through the pipette at 0.1 Hz, 1 Hz, and 3.3 Hz. Fle (1, 10 $\mu mol \cdot L^{-1}$) markedly prolonged the action potential duration measured at the level of 90 %, 50 %, and 20 %, without affecting either the resting membrane potential (RP) or the action potential amplitude (APA). The prolongation of APD by Fle was frequency-dependent. On washing-out of the drug from the bath, the action potential returned to the baseline.

Effects of Fle on APD were summarized in Tab 1. These observations suggested that Fle specifically acted on the current during the repolarization process of the action potential.

Effects on membrane currents When membrane potential was depolarized from a holding potential of −40 mV to +60 mV for 400 ms, the outward tail current was observed after the end of depolarizing command pulses (Fig 1). Fle markedly abolished the outward tail current after 400 ms voltage steps in a concentration- and rate-dependent fashion (Fig 2, 3). The most striking effect on the outward tail current was at 3.3 Hz stimulated frequency.

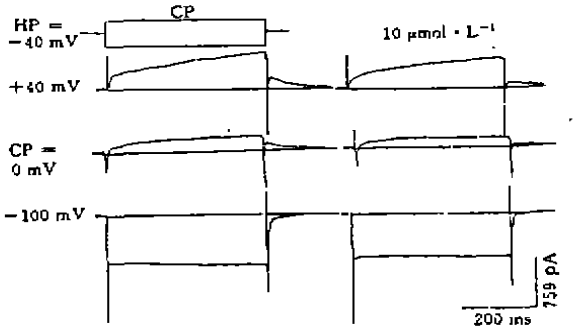

Fig 1. Effects of flecainide on membrane current of guinea pig left atrial cell. Holding potential −40 mV, duration 400 ms.

Tab 1. Effects of flecainide 10 $\mu mol \cdot L^{-1}$ on transmembrane potentials in the guinea pig left atrial single cell at different stimulated frequencies. $n=5$, $\bar{x}\pm s$. $^bP<0.05$, $^cP<0.01$ vs control.

	0.1 Hz		1 Hz		3.3 Hz	
	Control	Flecainide	Control	Flecainide	Control	Flecainide
−RP/mV	71±4	73±6	71±4	71±7	70±4	69±9
OS/mV	22±6	20±7	23±5	20±6	23±7	21±8
APA/mV	93±8	93±6	95±7	92±6	95±7	90±5
APD_{90}/ms	90±19	142±41[b]	104±22	172±45[b]	111±23	190±43[c]
APD_{50}/ms	55±20	81±40	67±23	101±47	73±24	116±49
APD_{20}/ms	28±10	41±21	35±14	54±27	39±15	62±27

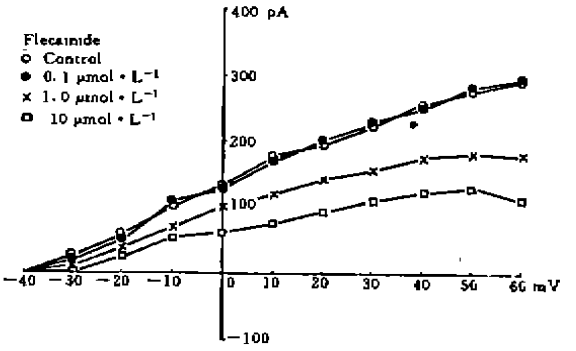

Fig 2. Effects of flecainide on current-voltage relation of tail current in guinea pig left atrial single cell. Holding potential −40 mV, duration 400 ms.

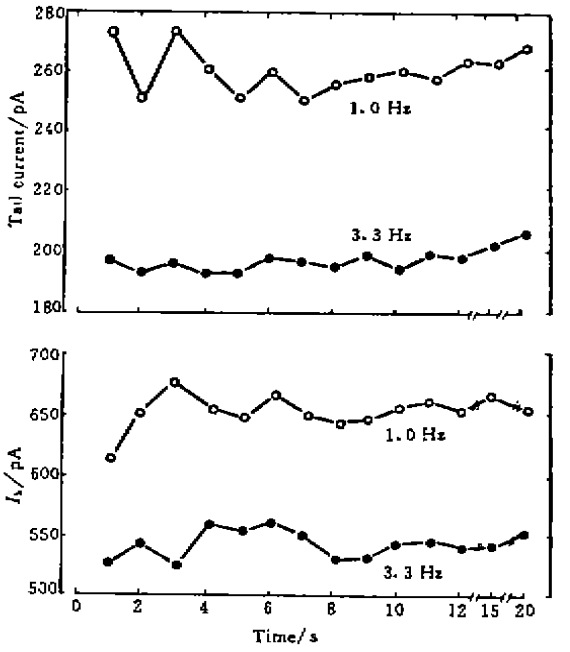

Fig 3. Effects of flecainide 10 μmol·L⁻¹ on tail current and I_{Ca} in guinea pig left atrial cell at 1 Hz and 3.3 Hz stimulation frequencies. Holding potential −40 mV, duration 400 ms.

The results indicated that Fle simply delayed the activation of time-dependent outward currents. Hence, the depolarizing pulses longer than 400 ms were used to examine its effects on the outward current. Fig 4 illustrated current records in response to the long voltage steps and the outward tail currents after 5000 ms. It was found also that Fle could depress the outward tail current in 400 — 5000 ms duration.

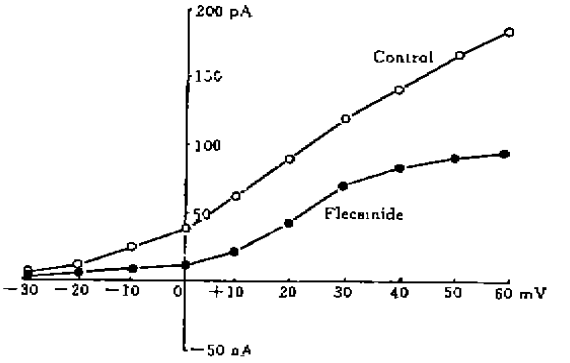

Fig 4. Effects of flecainide 10 μmol·L⁻¹ on tail current in long-lasting command pulse. Holding potential −40 mV, duration 5 s.

The effects of Fle on the outward tail current were examined in the presence of Co^{2+} 2 mmol · L^{-1} to block the I_{Ca}. The results showed that Fle significantly suppressed the amplitude of tail current to < 54 % of the control at all the activation voltages without influencing on the holding current in 4 cells. The results suggested that I_{Ca} did not contribute to the tail current.

Depression of outward tail current during command pulses between −30 mV to +60 mV was consistently observed. The magnitude of suppression of tail current was stronger at less positive potentials than that at negative ones. Following the steps to potentials +20 + 60 mV, the tail current were significantly abolished by the drug, with some release of block following the steps to more negative potentials. In 4 cells not exposed to Fle, the tail current amplitude did not decreased over an equal time interval. Hence, the reduction in tail current amplitude was

directly attributable to the drug, rather than to the spontaneous alternation or run-down of the current.

The recovery of the Fle effect was examined after 15 min of washing out. All of the cells recovered to 82 % — 94 % of the control. The cell was held at −40 mV, the delayed outward K current was evoked on depolarization and inward-rectifying I_{k1} current on hypopolarization. Effects of Fle on I_k were examined by applying depolarizing voltage steps to +60 mV and I_{k1} current hyperpolarizing voltage steps to −100 mV. The experiments were done with the presence of Co^{2+} 2 mmol·L^{-1} and the results were shown in Fig 5: I_k was markedly inhibited to 79.9 % at + 20 mV and 88.1 % at +60 mV with frequency-dependence (Fig 3). The I_{k1} current at −100 mV were decreased during Fle application. However, the recovery was obtained on the washing-out of the drug for 10 min.

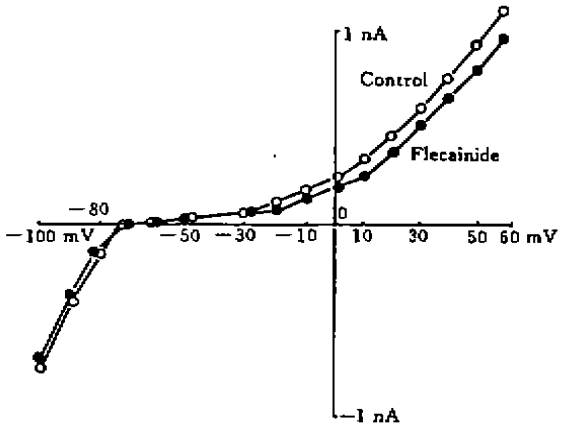

Fig 5. Effects of flecainide 10 μmol·L^{-1} on current-voltage relation of I_k and I_{k1} in guinea pig left atrial single cell. Holding potential −40 mV, duration 400 ms.

Since the voltage clamp pulses were applied from the holding potential of −40 mV in the present study, the effect of Fle on the holding current was examined in the presence of 2 mmol·L^{-1} Co^{2+}. This may give an information as to the Fle effect on the Na window current, since the current predominantly flowed at this voltage region. The amplitude of the holding current was +48±10 pA (n=6) in the control and it was +40±9 pA during the application of Fle for 10 min. There were no significant differences at different stimulated frequencies from 0.1, 1, to 3.3 Hz range between the values with or without Fle. Therefore, Fle had no significant effect on the holding current at −40 mV. the initial current-voltage relation measured at the peak of I_{Ca} showed that Fle 10 μmol·L^{-1} did not influence the peak of I_{Ca} in the guinea pig left atrial single cells.

DISCUSSION

The major findings of the present study were: Fle prologed the left atrial action potential (APD) in a rate-dependent fashion; The I_k and tail currents were specifically antagonisted by Fle in a rate-and voltage-dependent manner.

This characteristic prolongation of action potential induced by Fle could be well explained by the voltage-and rate-dependent inhibition of I_k and tail currents. The tail current represented current flow through the still open delayed rectifier channels in the repolarization, the I_k may play a major role in the late phase of the AP plateau and in the final repolarization. Accordingly, it could be proved that Fle influenced on the open delayed rectifier channels and the late phase of the AP plateau as well as the final repolarization. The effects of Fle on the action potentials and transmembrane ionic currents strongly suggested that the principal mechanism of action of this compound was to suppress the voltage-dependent potassium currents.

Fle exerted relatively little effect on membrane current flowing at negative potentials

from −30 mV to −100 mV in the voltage clamp cells. This indicated that Fle had little effect on the time-independent potassium current I_{k1}, which accounted for most of the current flowing this segment of membrane potentials. This was consistent with the lack of effect of Fle on the resting membrane potential.

Fle is a new antiarrhythmic agent that has been classified as class I_c[7,8] according to Vaugham-Willianms. However, our research proved that Fle was not completely consistent with the class I_c agents, as Fle markedly increased the APD in our experiment. Prolongation of the transmembrane action potential through inhibition of the time-dependent potassium current was an important feature of the class III antiarrhythmic agents. The accurate classification on Fle remains to be investigated.

REFERENCES

1 Grand B, Heuzey TJ, Perier P, Peronnerau P, Lavergane T, Hatem S. *et al.* Cellular electrophysiological effects of flecainide on human atrisl fibres. Cardiovase Res 1990; **24**: 232−8.

2 Somani P. Antiarrhythmic effects of flecainide. Clin Pharmacol Ther 1980; **27**: 464−70.

3 Usui M, Inoue H, Saihara S, Sugimoto T. Antifibrillatory effects of class III antiarrhythmic drug; comparstive study with flecainide. J Cardiovasc Pharmacol 1993; **21**: 376−83.

4 Anno T, Hondeghem LM. Interactions of flecainide with guinea pig cardiac sodium channels importance of activation unblocking to the voltage dependence of recovery. Circ Res 1990; **66**: 789−803.

5 Isenberg G, Klockner U. Calcium tolerant ventricular myocytes prepared by preincubation in a "KB medium." Pfluegers Arch 1982; **395**: 6−18.

6 Hiraoka M, Sawada K, Kawano S. Effects of quinidine on plateau currents of quinea pig ventricular myocytes: J Mol Cell Cardiol 1986; **18**: 1097−106.

7 Hellestrand KJ, Nathan AW, Bexton RS, Camm AJ. Electrophysiologic effects of flecainide acetae on sinus node function, anomalous atrio-ventricular connections and pacemaker thresholds. Am J Cardiol 1984; **53**: 30−38B.

8 Harrison DC, Meffin PJ, Winkle RA. Clinical Pharmacokinetics of antiarrhythmic drugs. Prog Cardiovas Dis 1977; **20**: 217−42.

28-32 3

氟卡尼电压和频率依赖性抑制豚鼠心房细胞迟发性外向钾电流

R965.2

杨宝峰, 杜智敏[1], 泽田光平[2]

(哈尔滨医科大学药理教研室, [1]哈尔滨医科大学临床药学研究所, 哈尔滨150086, 中国; [2]筑波研究所药理研究室, 筑波市, 茨城县300−26, 日本国)

A **摘要** 本文用单细胞钳法研究了氟卡尼(flecainide, Fle)对豚鼠心房单细胞膜电流的影响, 发现 Fle 频率依赖性延长动作电位时程, 并且浓度和频率依赖性减低迟发性外向钾电流和尾电流. Fle 对保持电流作用不显著, Fle 抗房性心律失常机制是电压依赖性抑制外向钾电流. Fle 显著影响复极过程, 故不符合 I_c 类抗心律失常药物.

关键词 氟卡尼; 心房; 电生理学