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Inhibitory Effects of the Antihistamines Epinastine, Terfenadine, and Ebastine on Potassium Currents in Rat Ventricular Myocytes

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Abstract

We examined and compared the inhibitory effects of three non-sedating antihistamines, terfenadine, ebastine, and epinastine, on delayed rectifier potassium current (I_K) and transient outward potassium current (I_{to}) of rat isolated ventricular myocytes, using a patch clamp technique.

Terfenadine, ebastine and epinastine were found to inhibit I_K with IC50 values of 5·96, 15·3 and 145 μM, respectively. I_{to} was suppressed by epinastine with an IC50 value of 69·5 μM. The order of arrhythmogenicity, assessed by the inhibition of I_K, was ranked as terfenadine > ebastine > epinastine, consistent with that of the potencies of each drug for QT prolongation reported in rats.

Terfenadine is a non-sedating antihistamine widely used clinically. While its antihistaminergic action is attributed to its active metabolite fexofenadine (Garteiz et al 1982), terfenadine itself possesses a potent arrhythmogenic activity (Woosley et al 1993). Fatal ventricular arrhythmias, including torsades de pointes have been reported to be evoked when terfenadine is either administered far in excess of the ordinary dose (Davies et al 1989), concomitantly administered with a metabolic inhibitor (Mathews et al 1991; Monahan et al 1991), or administered to patients with risk factors such as hepatic dysfunction (Kamisako et al 1995). Ebastine, which is structurally similar to terfenadine, and epinastine, which is mainly excreted unmetabolized into the urine, are free from being implicated in any clinical cases of arrhythmia, indicating that these agents may be used as alternatives to terfenadine. However, ebastine is reported to cause QTc prolongation when administered intravenously to guinea-pigs at a relatively high dose (Hey et al 1996). We have reported the arrhythmogenicity of ebastine, albeit less potent than terfenadine, by analysing the relationship between its plasma concentration and the change in QT interval during constant intravenous infusion to rats (Ohtani et al 1999). In contrast, no arrhythmogenic activity has been reported for epinastine. Therefore, the rank order of arrhythmogenicity is presumed to be terfenadine > ebastine > epinastine.

The arrhythmogenic action of terfenadine has been attributed to its inhibitory effect on cardiac potassium currents (Rampe et al 1993; Woosley et al 1993). Ebastine has also been reported to suppress cardiac potassium currents, although less potently than terfenadine (Ko et al 1997), which is in good agreement with our previous in-vivo findings for QT prolongation (Ohtani et al 1999). However, the effects of epinastine on potassium currents have not been evaluated. Moreover, no study has been performed in the same animal species to systematically correlate the electrocardiographic potency for QT prolongation in-vivo with the electrophysiological potency for the inhibition of potassium currents of ventricular myocytes in-vitro. Thus, we report on the inhibitory potencies of terfenadine, ebastine and epinastine, for which we have previously examined the relationships between plasma concentration and QT prolongation (Ohtani et al 1997, 1999), on delayed rectifier

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potassium current (I_K) and transient outward potassium current (I_{to}) in rat isolated ventricular myocytes, the same species as used in-vivo.

Materials and Methods

Materials

Terfenadine, ebastine and epinastine were kindly provided by Marion Merrell Dow K. K. (Osaka, Japan), Dainippon Pharmaceuticals Co. Ltd (Osaka, Japan), and Nippon Boehringer Ingelheim (Kawanishi, Japan), respectively. Tetraethylammonium (TEA) and 4-aminopyridine were purchased from Tokyo Chemical Industry Co., Ltd (Tokyo, Japan) and Wako Pure Chemical Industries, Ltd (Osaka Japan), respectively. All other agents used were of reagent or HPLC grade. Deionized, distilled water was used throughout and was purified by use of Milli-Q system (Millipore, MA). Epinastine was dissolved in water, terfenadine and ebastine were dissolved in ethanol. Final ethanol content in the experimental solution was less than 0·1% (v/v) and it was confirmed that the solvent alone did not affect K^+ channels.

Cell preparation

Isolated ventricular myocytes were enzymatically prepared from rats as described previously (Watanabe et al 1996). Briefly, male Sprague–Dawley rats, 220–370 g, were anaesthetized with pentobarbital sodium (50 mg kg^{-1}, i.p.) and artificially ventilated with a respirator. The heart was removed from the thoracic cavity, mounted on a Lagendorff's perfusion system, and was retrogradely perfused with oxidized HEPES–Tyrode solution at 37°C for 5 min. The perfusate was then changed to a nominally Ca^{2+}-free Tyrode solution, followed by a Ca^{2+}-free Tyrode solution containing 300 mg L^{-1} collagenase for 25–32 min. After enzymatic digestion, the heart was perfused with a high K^+, low Cl^- solution (modified Kraftbrühe (KB) solution). Ventricular tissue was minced into small pieces and gently agitated in the modified KB solution to isolate cells. The suspension was filtered through a stainless-steel filter (100 μm-pore) and stored at 4°C. The composition of the HEPES–Tyrode solution was (in mM): NaCl 134; KCl 5·4; $CaCl_2$ 1·8; $MgCl_2$ 0·5; $NaHCO_3$ 0·33; glucose 5·5; HEPES 5·0, pH 7·4, adjusted by addition of NaOH. The Ca^{2+}-free Tyrode solution was prepared by omitting $CaCl_2$ from the HEPES–Tyrode solution. The composition of the modified KB solution was (in mM): KOH 70; L-glutamic acid 50; KCl 40; taurine 20; KH_2PO_4 20; $MgCl_2$ 3·0; glucose 10; EGTA 1·0; HEPES 10.

Electrophysiological recording

Whole-cell membrane currents were recorded by a patch clamp technique. Isolated cells were placed in a recording chamber (1·0 mL volume) and perfused, at a rate of 3 mL min^{-1}, with a modified HEPES–Tyrode solution containing 3·0 mM of $CoCl_2$ to suppress Ca^{2+} current.

Glass patch electrodes with a tip diameter of 2–3 μm were made by a vertical puller (Model PB-7, Narishige, Tokyo) and filled with an internal solution. The internal solution consisted of (in mM): potassium L-aspartate 110; KCl 20; $MgCl_2$ 1·0; potassium ATP 5·0; potassium phosphocreatine 5·0; EGTA 10; HEPES 5·0, pH 7·4, adjusted by addition of KOH. The pipette resistance ranged from 2 to 4 MΩ when filled with the internal solution. The free Ca^{2+} concentration in the pipette solution was adjusted to pCa 8 (Fabiato & Fabiato 1979; Tsien & Rink 1980).

The electrode was connected to a patch-whole cell clamp amplifier (Nihon Kohden CEZ-2300). Voltage command pulses were generated and data were acquired on a personal computer (Compaq Prolinea 4/50, TX) using the pCLAMP software package (Axon Instruments, CA). Current signals were digitized with a sampling interval of 2 kHz and stored on the computer hard disk. Correction was made for a junctional potential of −12 mV between the internal solution and perfusate.

The delayed rectifier potassium current (I_K) and the transient outward potassium current (I_{to}) were recorded according to the method of Slawsky & Castle (1994). The membrane potential was held at −90 mV and a depolarizing pulse to +40 mV for 200 ms was applied following a pre-pulse to −20 mV for 15 ms to inactivate the Na^+ current. I_K was recorded with the perfusate containing 3 mM 4-aminopyridine to suppress I_{to}. Recording of I_{to} was performed with a modified HEPES–Tyrode solution substituted with 80 mM TEA in place of the stoichiometrically equivalent NaCl to suppress I_K. The amplitudes of I_K and I_{to} were assessed by the method of Slawsky & Castle (1994). I_{to} was measured as the integral of the outward current with respect to the 'steady-state' current remaining at the end of the depolarizing pulse. I_K was defined as the amplitude of the remaining current at the end of the depolarizing pulse. Drug responses were examined by applying perfusates containing several concentrations of a test drug for at least 5 min to ensure stabilization of the drug effect. Not more than three concentrations of a drug were applied to

an individual preparation to preserve the stability of the myocytes. After examining the drug effects, the perfusate, containing both 80 mM TEA and 3 mM 4-aminopyridine, was applied in order to evaluate the maximum blockade of I_K or I_{to}. The inhibitory effect of the drug was normalized by use of the above maximum blockade.

IC50 values were obtained by fitting the mean values of the concentration–inhibition relationship to equation 1 using a non-linear least-squares regression method.

$$\text{Current amplitude} = 1 - C/(IC50 + C) \quad (1)$$

where C represents the concentration of the tested drug.

Results and Discussion

Exposure of rat ventricular myocytes to terfenadine, ebastine or epinastine resulted in a concentration-dependent suppression of the delayed rectifier potassium current (I_K) with IC50 values of 5·96, 15·3, and 145 μM, respectively (Figure 1). Terfenadine and ebastine did not suppress the transient outward current (I_{to}) even at concentrations up to 10 μM. Epinastine, however, suppressed I_{to} at the higher concentrations in the range, with an IC50 value of 69·5 μM (Figure 2).

Epinastine inhibited I_K and I_{to} of rat ventricular myocytes at concentrations higher than those observed clinically. This weak inhibitory potency for I_K may be essential in order to avoid arrhythmogenicity. Although it remains unclear whether suppression of I_{to} leads to arrhythmogenicity, the inhibitory effect of epinastine on I_{to} may not lead to its clinical arrhythmogenicity since the potency for I_{to} was far weaker when compared with its clinical concentration. Additionally, epinastine is mainly eliminated unmetabolized via renal excretion (Azuma et al 1992) and thus it would appear to be relatively unsusceptible to metabolic drug interactions that would cause an increase in its plasma concentration in the gut and/or liver. Therefore, the data presented here for epinastine may not be directly relevant to its possible clinical arrhythmogenicity.

In this study, terfenadine suppressed I_K in rat ventricular myocytes. Terfenadine has been reported to inhibit the I_K of various experimental preparations such as isolated feline myocytes

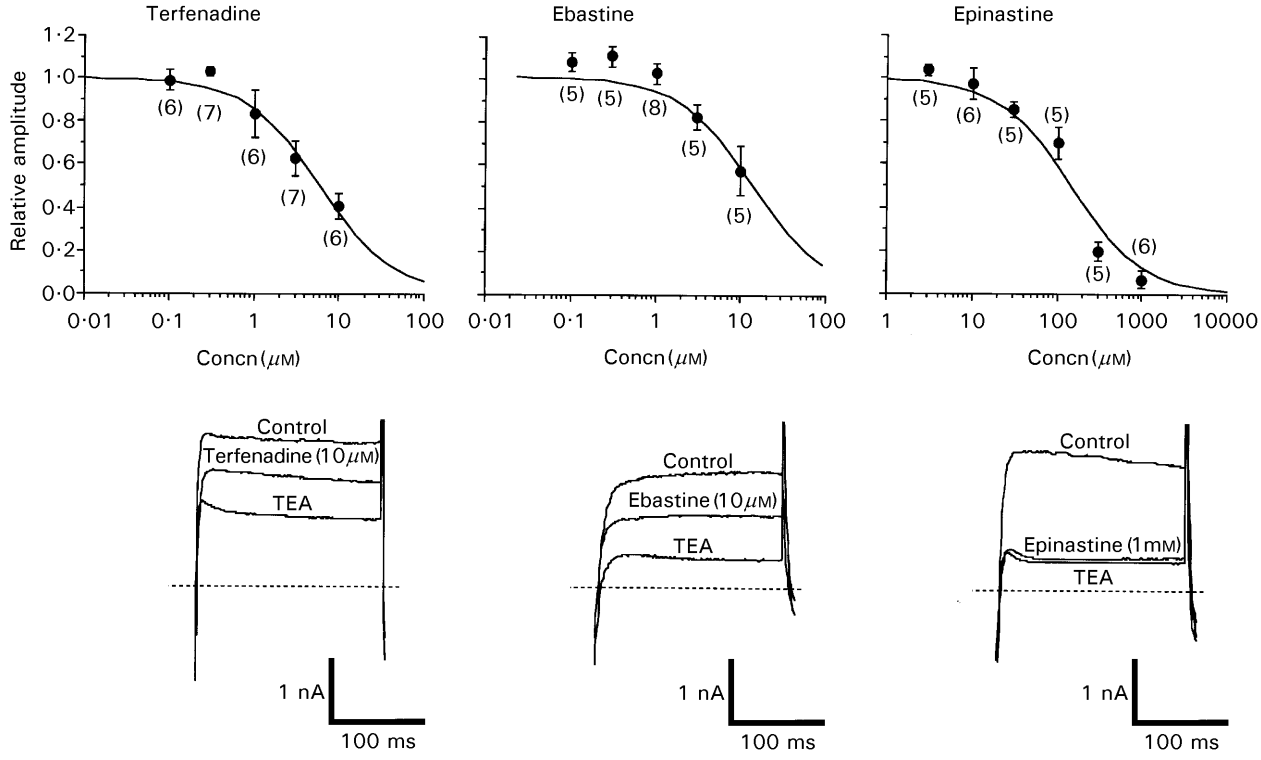

Figure 1. Concentration–response curves for inhibitory effects of terfenadine, ebastine, and epinastine on delayed rectifier potassium current (I_K) in rat ventricular myocytes. Each point and vertical bar represents the mean ± s.e.m. The number of experiments for each point is shown in parentheses. The solid lines were obtained by fitting equation 1 to the observed data. Original current traces in control and after exposure to either antihistamines or tetraethylammonium (TEA) are presented in the lower part of the figure. The current was elicited by a 200-ms depolarizing pulse to +40 mV from a holding potential of −90 mV following a 15-ms prepulse to −20 mV. The external solution contained 3 mM 4-aminopyridine.

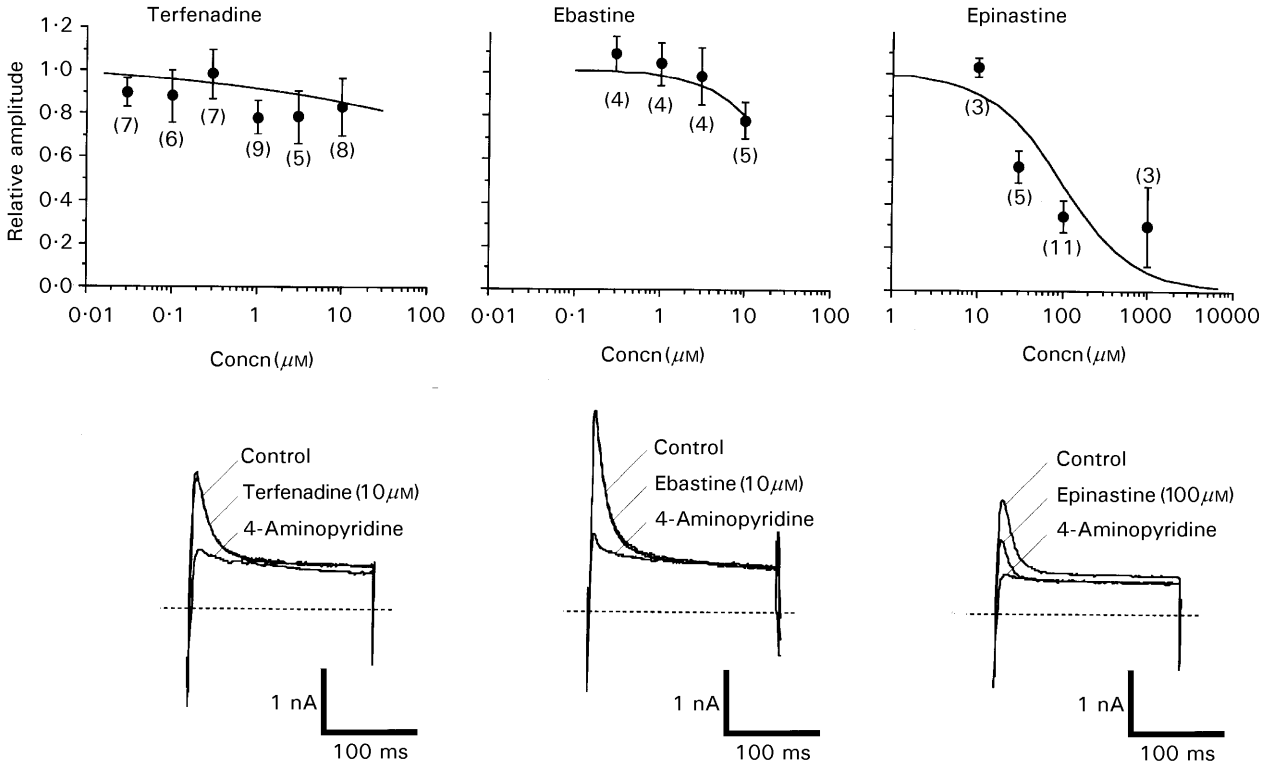

Figure 2. Concentration–response curves for the inhibitory effects of terfenadine, ebastine, and epinastine on transient outward potassium current (I_{to}) in rat ventricular myocytes. Each point and vertical bar represents the mean ± s.e.m. The number of experiments for each point is shown in parentheses. The solid lines were obtained by fitting equation 1 to the observed data. Original current traces in control and after exposure to either antihistamines or 4-aminopyridine are presented in the lower part of the figure. The current was elicited by a 200-ms depolarizing pulse to +40 mV from a holding potential of −90 mV following a 15-ms prepulse to −20 mV. The external solution contained 80 mM tetraethylammonium (TEA).

(Woosley et al 1993), human embryonic kidney cells expressing a K^+ (fHK) channel cloned from human heart (Rampe et al 1993), isolated guinea-pig myocytes (Ducic et al 1997), and *Xenopus* oocytes expressing the cloned *HERG* channel (Ducic et al 1997; Ko et al 1997). The I_K of rat cardiac myocytes is reported to differ from that observed in man or guinea-pig (Apkon & Nerbonne 1991; Deal et al 1996). Although the inhibitory potency of terfenadine on I_K in rat ventricular cells was less potent relative to that observed in previous preparations, it is now clear that the agent inhibits the I_K of a wide variety of animal species including rats. Ebastine was also reported by Ko et al (1997) to suppress *HERG*-type rapid delayed rectifier potassium currents (I_{Kr}) with an equivalent potency to, but a weaker efficacy than terfenadine. In this study, concentrations of ebastine higher than 30 μM were not experimentally feasible due to its insolubility in water, thus the inhibitory efficacy was not precisely determined. In any case, the inhibitory potency of ebastine on the I_K of rat ventricular myocytes was shown to be three times less potent than that of terfenadine.

In contrast to I_K, I_{to} has been reported to be virtually insusceptible to terfenadine and ebastine (Crumb & Brown 1993; Ko et al 1997). Consistent with those previous findings, neither terfenadine nor ebastine significantly suppressed I_{to} (Figure 2).

We comparatively and qualitatively investigated the effects of terfenadine, ebastine, and epinastine on the potassium currents of rat ventricular myocytes in-vitro. The order of inhibitory potency for I_K, which is considered to play the major role in QT prolongation (Zehender et al 1991), was ranked to be terfenadine > ebastine > epinastine. This correlates well with that of the potency for QT prolongation in-vivo demonstrated by our pharmacokinetic–pharmacodynamic analysis of rat electrocardiograms (Ohtani et al 1997, 1999).

Terfenadine is known to bind plasma protein avidly, with the plasma unbound fraction being only 3% in man and even less in rats (Arky 1998). Thus it follows that there is a discrepancy between the effective concentrations when comparing conditions in-vivo and in-vitro because pharmacological effects are attributed to unbound, not total drug concentrations. Plasma terfenadine concentrations are reported to be 90–120 nM during the event of torsades de pointes in man (Davies et al 1989; Monahan et al 1991), which correspond to plasma unbound concentrations of 2·7–3·6 nM. The

IC50 values of terfenadine for inhibition of cardiac potassium currents range from 50 to 400 nM (Rampe et al 1993; Woosley et al 1993; Salata et al 1995; Ko et al 1997), providing a 20- to 100-fold concentration than that of the unbound drug at the events.

Possible explanations for the above discrepancy include inter-species differences and/or changes in the electrophysiological properties of arrhythmogenicity-related ion channels under in-vitro conditions, especially those encountered in a gene-expression system. Although enzymatically isolated ventricular myocytes from the same animal species were used in this study to maintain the ion channel in an intact form as much as possible, the discrepancy still remains. Terfenadine is considered to act as an open-channel blocker for the potassium channel and is considered not to directly access the channel pore from the exterior (Rampe et al 1993). Therefore a different permeability to myocytes and/or the use dependency of blockade may constitute another explanation for the discrepancy in free concentration between in-vivo and in-vitro data. It is necessary to consider this discrepancy to quantitatively deduce the clinical arrhythmogenic risks of antihistamines from their electrophysiological properties obtained in-vitro.

In conclusion, the rank order of inhibitory potency of terfenadine, ebastine, and epinastine for I_K was in agreement with that of the potency for QT prolongation in-vivo. Epinastine suppressed I_K and I_{to} of rat ventricular myocytes at a concentration far above that of the clinical range. Further studies are necessary to resolve the in-vitro vs in-vivo quantitative discrepancy of their potency on a free-drug concentration basis.

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