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Mefenamic acid as a novel activator of L-type voltage-dependent Ca^{2+} channels in smooth muscle cells from pig proximal urethra

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1 The effects of mefenamic acid and Bay K 8644 on voltage-dependent nifedipine-sensitive inward Ba^{2+} currents in pig urethral myocytes were investigated by use of conventional whole-cell configuration patch clamp.

2 Mefenamic acid increased the peak amplitude of voltage-dependent nifedipine-sensitive inward Ba^{2+} current without shifting the position of the current–voltage relationship.

3 Mefenamic acid (300 μM) caused little shift in the activation curve although the voltage dependence of the steady-state inactivation was shifted to more positive potentials by 11 mV in the presence of mefenamic acid.

4 Bay K 8644 ($\geqslant$100 nM) enhanced voltage-dependent nifedipine-sensitive inward Ba^{2+} currents in a concentration- and voltage-dependent manner, shifting the maximum of the current–voltage relationship by 10 mV in the hyperpolarizing direction.

5 Bay K 8644 (1 μM) significantly shifted the voltage dependence of the activation curve to more negative potentials by approximately 9 mV although Bay K 8644 caused little shift in the steady-state inactivation curve.

6 These results indicate that mefenamic acid increased voltage-dependent nifedipine-sensitive inward Ba^{2+} currents through the activation of L-type Ca^{2+} channels with different kinetics from those of Bay K 8644 in pig urethral myocytes.
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Abbreviations: 4-AP, 4-aminopyridine; BK_{Ca} channels, large conductance Ca^{2+}-activated K^+ channels; DMSO, dimethyl sulphoxide; E_{rev}, reveasal potential for Ba^{2+}; K_{ATP} channel, ATP-sensitive K^+ channel; PSS, physiological salt solution; STOCs, spontaneous transient outward currents; TEA^+, tetraethylammonium

Introduction

Fenamates (mefenamic acid, flufenamic acid, niflumic acid, etc) have been used as nonsteroidal anti-inflammatory drugs in clinical practise. Mefenamic acid is one of the most commonly used fenamates, not only as an anti-inflammatory agent but also in the therapy of patent ductus arteriosus in preterm infants. Mefenamic acid is known to affect various types of membrane channel in smooth muscles. Mefenamic acid has been shown to activate Ca^{2+}-independent delayed rectified K^+ currents (canine jejunum, Farrugia *et al*., 1993), large conductance Ca^{2+}-activated K^+ channels (i.e. BK_{Ca} channels; pig coronary artery, Ottolia & Toro, 1994) and α_1-adrenoceptor-activated nonselective cation currents (rabbit portal vein, Yamada *et al*., 1996). Recently, we have directly demonstrated the effects of mefenamic acid on several types of K^+ channels in pig urethra by use of single-channel recordings (Teramoto *et al*., 2003). In this tissue, mefenamic acid increased the activity of BK_{Ca} channels and ATP-sensitive K^+ channels (K_{ATP} channels) and decreased the activity of 4-aminopyridine (4-AP)-sensitive K^+ channels. Additionally, mefenamic acid ($\leqslant$30 μM) activated spontaneous transient outward currents (STOCs) although at higher concentrations ($\geqslant$100 μM) it increased sustained outward currents, diminishing the activity of STOCs. We have thus concluded that mefenamic acid possesses multiple effects on outward K^+ currents (Teramoto *et al*., 2003). Although mefenamic acid clearly possesses a dual (agonistic and antagonistic) action on membrane currents (Teramoto *et al*., 2003), its effects on inward currents still remain to be elucidated. The purposes of the present study were to investigate the effects of mefenamic acid on voltage-dependent inward Ba^{2+} currents, comparing the actions of Bay K 8644, a well-known voltage-dependent L-type Ca^{2+} channel agonist, on Ca^{2+} current kinetics in pig urethral myocytes.

Methods

Cell preparation and recording procedure

Fresh female pig urethra with attached urinary bladder was collected from a local abattoir. Smooth muscle was dissected from the proximal urethra, 1–2 cm distal to the bladder neck.

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We employed the cell dispersion method (the gentle tapping method) previously described (Teramoto & Brading, 1996). The set-up of the patch-clamp experimental system used was essentially the same as described previously (Teramoto *et al*., 2003). All experiments were performed at room temperature (21–23°C).

Solutions and drugs

For whole-cell recordings, the ionic composition of the physiological salt solution (PSS) in the bath was (mM): Na^+ 140, K^+ 5, Mg^{2+} 1.2, Ca^{2+} 2, Cl^- 151.4, glucose 10, HEPES 10, and it was titrated to pH 7.35–7.40 with Tris base. For recording whole-cell currents, high caesium pipette solution contained (mM): Cs^+ 130, tetraethylammonium (TEA^+) 10, Mg^{2+} 2, Cl^- 144, glucose 5, EGTA 5, ATP 5, HEPES 10/Tris (pH 7.35–7.40). Ba^{2+} 10 mM bath solution contained (mM): Ba^{2+} 10, TEA^+ 135, Cl^- 155, glucose 10, HEPES 10/Tris (pH 7.35–7.40). Cells were allowed to settle in the small experimental chamber (approximately 80 μl in volume). The bath solution was superfused by gravity throughout the experiments at a rate of 2 ml min^{-1}. All chemicals were purchased from Sigma (Sigma Chemical K.K., Tokyo, Japan). The stock solution of mefenamic acid (300 mM) was made daily by dissolving it in dimethyl sulphoxide (DMSO). This was diluted in the bath solution (final concentration, 300 μM) and was further ultrasonicated for about 15–25 min until it was dissolved (ultrasonicator, Bransonic, Branson Ultrasonic Corporation, U.S.A.). The final concentration of DMSO was less than 0.5%, and did not affect membrane currents.

Data analysis

The whole-cell current data were low-pass filtered at 500 Hz (−3 dB) by an 8 pole Bessel filter (3611 multifunction filter, NF Electronic Instruments, Yokohama, Japan), sampled at 1 ms and analysed on a computer (Macintosh G4, Apple Computer Japan Ltd, Tokyo, Japan) by use of the commercial software 'Mac Lab 3.5.6' (ADInstruments Pty Ltd, Castle Hill, Australia).

Conditioning pulses of various amplitudes were applied (up to +30 mV, 8 s duration) before application of the test pulse (to +10 mV, 1 s duration). An interval of 20 ms was allowed between these two pulses to estimate possible contamination of the capacitive current. The peak amplitude of Ba^{2+} current evoked by each test pulse was measured before and after application of drugs. The lines were draw by fitting the data to the following equation in the least-squares method,

$$I = (I_{max} - C)/\{1 + \exp[(V - V_{half})/k]\} + C$$

where I, I_{max}, V, V_{half}, k and C are the relative amplitude of Ba^{2+} inward currents observed at various conditioning voltages (I) and after conditioning at −90 mV (I_{max}), voltage of the conditioning pulse (V), and its voltage when the amplitude of Ba^{2+} inward current was reduced to half (V_{half}), the slope factor (k) and the fraction of the noninactivating component of Ba^{2+} inward current (C). A −90 mV conditioning pulse was applied to achieve the value of I_{max}.

Activation curves were derived from the current–voltage relationships. Conductance (G) was calculated from the equation $G = I_{Ba}/(E_m - E_{rev})$, where I_{Ba} is the peak current elicited by depolarizing test pulses from −50 to 40 mV from a holding membrane potential of −50 mV and E_{rev} is the reversal potential for Ba^{2+}. G_{max} is the maximal Ba^{2+} conductance (calculated at potentials above 10 mV). The points for G/G_{max} were plotted against the membrane potential as relative amplitude.

Statistical analysis

Statistical analyses were performed with analysis of variance (ANOVA) test (two-factor with replication). Changes were considered significant at $P<0.05$ (*). Data are expressed as mean with the standard deviation (s.d.).

Results

Effects of mefenamic acid on voltage-dependent Ba^{2+} inward currents

The effects of mefenamic acid on voltage-dependent Ca^{2+} currents were investigated by use of conventional whole-cell configuration patch clamp experiments. When the bath solution was PSS, the peak amplitude of inward Ca^{2+} current was too small to analyse (less than 20 pA at 0 mV). In order to enhance the inward currents for reasonable analysis and to isolate voltage-dependent inward currents through Ca^{2+} channels by inhibiting other Ca^{2+}-activated mechanisms (such as Ca^{2+}-activated K^+ currents and Ca^{2+}-activated Cl^- currents, etc), 10 mM Ba^{2+} bath solution containing 135 mM TEA^+ was used and the pipette was filled with a Cs^+-TEA^+ solution containing 5 mM EGTA (Teramoto & Brading, 1998; Teramoto *et al*., 2001). Figure 1a shows the time course of the effects of mefenamic acid (300 μM) on the voltage-dependent Ba^{2+} inward current evoked by a depolarizing pulse of +10 mV from a holding potential of −50 mV. The depolarizing pulses were applied every 20 s. When the peak amplitude of voltage-dependent Ba^{2+} currents just before application of mefenamic acid (control) was taken as one, mefenamic acid slightly but significantly enhanced I_{Ba} in a concentration-dependent manner (100 μM, 1.1 ± 0.03, $n=8$; 300 μM, 1.3 ± 0.06, $n=6$). Note that the number of observations indicates the total cell number from each different pig. On removal of mefenamic acid, the peak amplitude gradually recovered to the control level. Additional application of 10 μM nifedipine completely suppressed voltage-dependent Ba^{2+} currents, and Cd^{2+} (100 μM) did not further change their amplitude.

To measure the current–voltage relationship, depolarizing step pulses (1 s duration) were applied in 10 mV increments from −40 to +40 mV from a holding potential of −50 mV. At potentials more positive than −30 mV, voltage-dependent Ba^{2+} current was evoked (Figure 2), which reached a peak and then gradually decayed. The maximum peak amplitude was obtained at approximately +10 mV and the amplitude was reduced at more positive potentials. Voltage-dependent Ba^{2+} current was enhanced by mefenamic acid (300 μM) at potentials more positive than −20 mV. At −30 mV, both the peak amplitude and the amplitude at the end of the command pulse were smaller, but the time course of the current decay was identical in the absence and presence of mefenamic acid (Figure 2a(i), (ii)). An experiment is illustrated in Figure 2b, which compares the current–voltage relationships in control

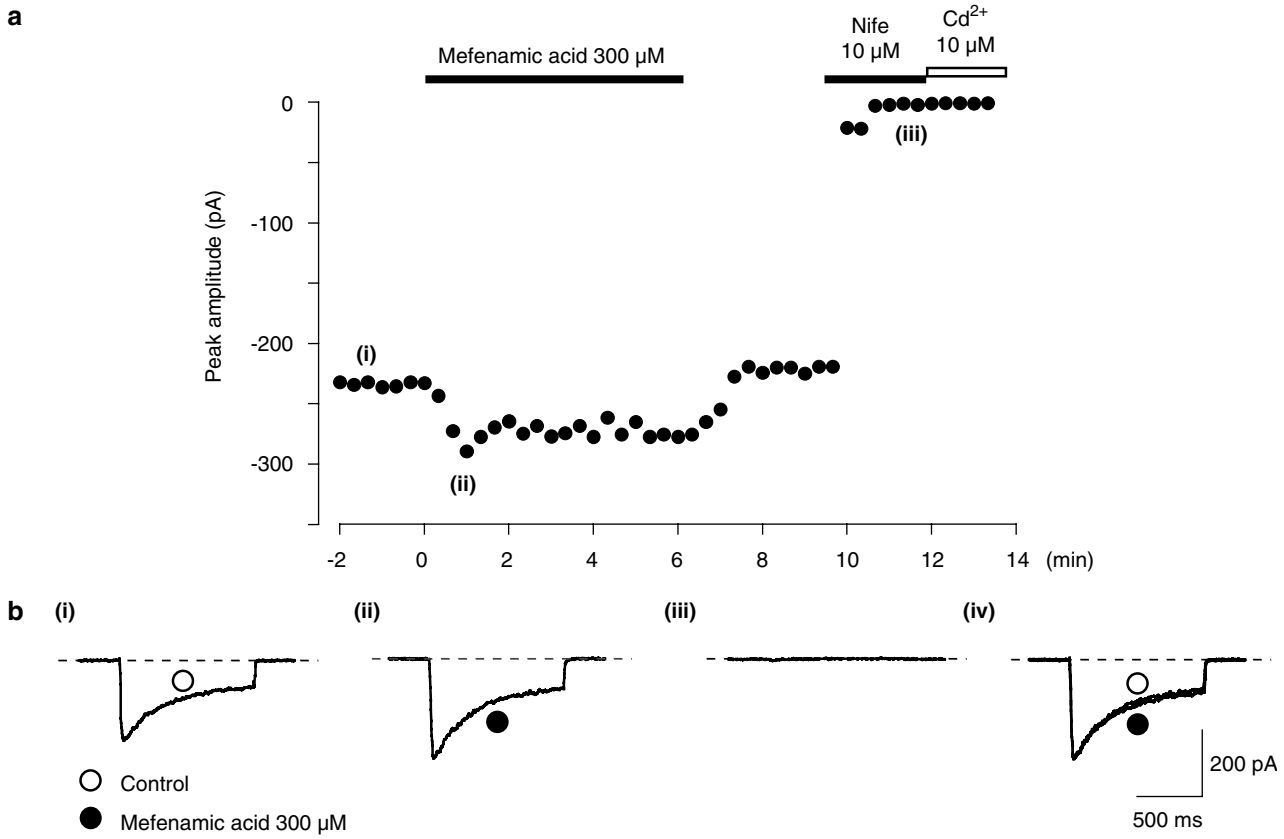


Figure 1 Effects of mefenamic acid (300 μM) on voltage-dependent Ba^{2+} currents using conventional whole-cell recording from an isolated pig urethral myocyte. (a) The time course of activation of the peak amplitude of voltage-dependent Ba^{2+} currents by mefenamic acid (300 μM) is shown. Inward currents were elicited by voltage steps (1 s duration) to +10 mV from a holding potential of −50 mV every 20 s. Time 0 indicates the time when mefenamic acid was applied. The activation produced by mefenamic acid was reversed by washing with drug free solution. The application of 10 μM nifedipine suppressed voltage-dependent Ba^{2+} currents. No further inhibition was observed by additional application of Cd^{2+} (100 μM). (b) The four traces show inward currents, elicited by voltage steps in control solution (i), after the application of mefenamic acid (ii) and in the presence of nifedipine (iii). (iv) When the currents were scaled to have the same peak amplitudes and superimposed in the absence and presence of mefenamic acid (300 μM), it can be seen that their time courses were unchanged.

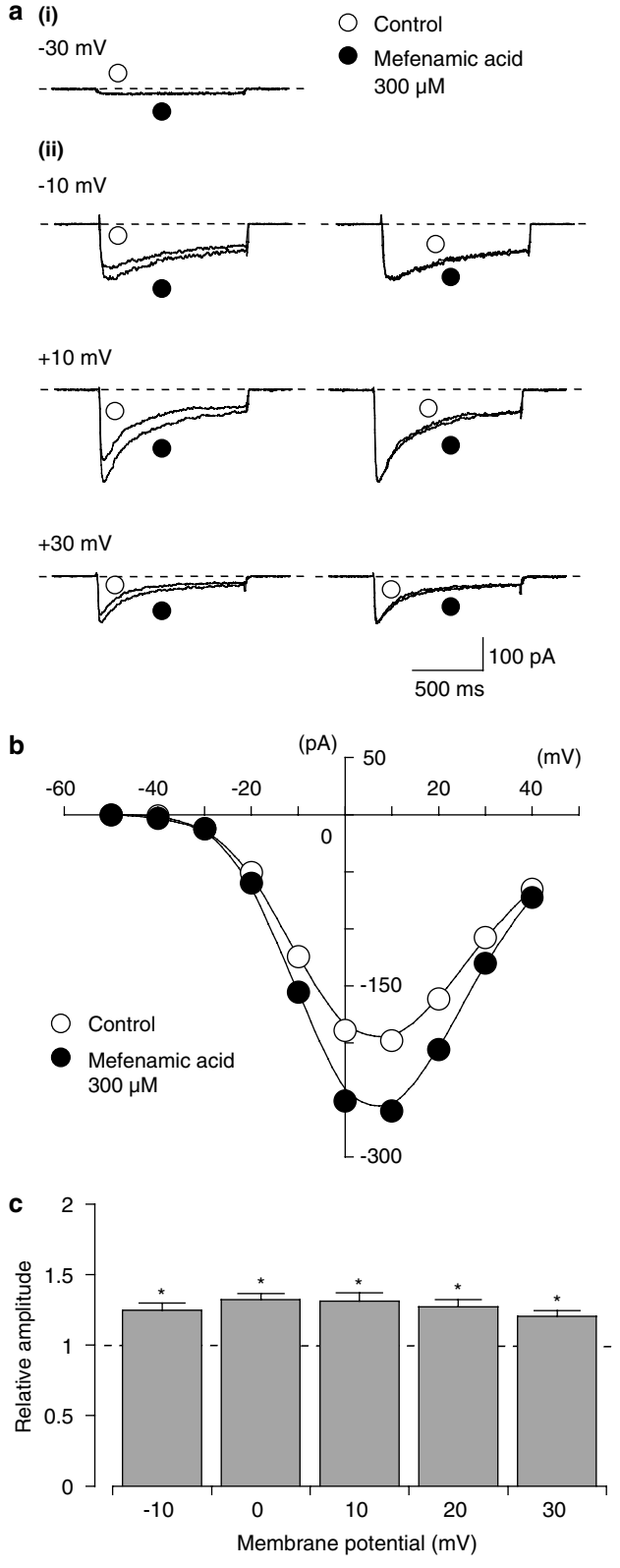
solution and 5 min after mefenamic acid was applied to the bath. As shown in Figure 2c, the activation by mefenamic acid shows little voltage-dependency between −10 mV and +30 mV.

Effects of Bay K 8644 on voltage-dependent Ba^{2+} inward currents

Figure 3a shows the time course of the effects of Bay K 8644 (1 μM) on voltage-dependent Ba^{2+} currents evoked by depolarizing pulses of +10 mV from a holding potential of −50 mV. The depolarizing pulses (1 s duration) were applied every 20 s. When the peak current amplitude just before application of Bay K 8644 (control) was taken as one (Figure 3b(i)), Bay K 8644 greatly enhanced the voltage-dependent Ba^{2+} currents (Figure 3b(ii), $n=6$). Additional application of 10 μM nifedipine suppressed the currents. Figure 3c shows that Bay K 8644 ($\leqslant$10 μM) increased the peak amplitude of voltage-dependent Ba^{2+} currents in a concentration-dependent manner. Figure 4a shows the current–voltage relationships in the absence and presence of 1 μM Bay K 8644. Bay K 8644 increased the peak amplitude of voltage-dependent Ba^{2+} currents evoked by depolarizing pulses (1 s duration) from a holding potential of −50 mV at levels more positive than −30 mV, shifting the maximum of the current–voltage relationship by 10 mV in the hyperpolarizing direction. The activation showed some voltage-dependency (Figure 4b).

The stimulating effects of mefenamic acid and Bay K 8644 were analysed by determining the steady-state inactivation and activation curves for voltage-dependent Ba^{2+} currents. This voltage-dependent inactivation was investigated before and after application of mefenamic acid and Bay K 8644 using the experimental protocol shown in Figure 5 (conditioning pulse duration, 8 s; holding membrane potential, −90 mV). In the absence of activators (control), inactivation of voltage-dependent Ba^{2+} currents occurred with conditioning pulses positive to −50 mV. In the presence of 300 μM mefenamic acid (approximately 5 min later), the voltage-dependent inactivation curve in the same cells was shifted to the right (Figure 5a). The 50% inactivation potentials, evaluated by means of Boltzmann fitting, were −31 mV (control; -30 ± 2 mV, $n=4$) and −19 mV (mefenamic acid; -18 ± 2 mV, $n=4$, $P<0.05$), respectively. On the other hand, 1 μM Bay K 8644 slightly

shifted the steady-state inactivation curve to the left (Figure 5b). The 50% inactivation potentials, evaluated by means of Boltzmann fitting, were −29 mV (control; −29±2 mV, $n=4$) and −32 mV (Bay K 8644; −32±1 mV, $n=4$, $P<0.05$), respectively.



The activation curves obtained from the current–voltage relationships in Figures 2b and 4a, fitted to the Boltzmann equation, are shown in Figure 5. Mefenamic acid (300 μM) caused little shift of the activation curve (the 50% activation potentials; −6 mV (control; −6±2 mV, $n=4$) vs −6 mV (mefenamic acid; −6±2 mV, $n=4$, $P<0.05$, Figure 5a). On the contrary, in Figure 5b, Bay K 8644 (1 μM) shifted the curve to the left, significantly decreasing the 50% activation potentials from −8 mV (control; −7±2 mV, $n=4$) to −17 mV (Bay K 8644; −17±2 mV, $n=4$, $P<0.05$).

Discussion

The present study provides the first direct electrophysiological evidence that mefenamic acid, an anti-inflammatory agent, is an activator of L-type Ca^{2+} channels in smooth muscle.

Properties of voltage-dependent Ca^{2+} channels in urethra

A number of studies have demonstrated that urethral myogenic tone is critically dependent on the influx of Ca^{2+} across the cell membrane, since removal of external Ca^{2+} or inhibition by various types of Ca^{2+} channel blockers reduces the urethral tone significantly in rats, humans and pigs *in vitro* (reviewed by Brading, 1999; Teramoto *et al*., 2001). Voltage-dependent Ca^{2+} currents have been widely demonstrated in urethral myocytes by use of whole-cell recordings (sheep, Cotton *et al*., 1997; pig, Teramoto & Brading, 1998; human, Hollywood *et al*., 2003; rabbit, Bradley *et al*., 2004). Some urethral myocytes (human, Hollywood *et al*., 2003; rabbit, Bradley *et al*., 2004) possess two types of voltage-dependent Ca^{2+} channels with different biophysical and pharmacological properties, namely L-type and T-type Ca^{2+} channels. However, we have previously reported that L-type Ca^{2+} channels are probably the only type of voltage-dependent Ca^{2+} channel present in pig urethra, as assessed by several electrophysiological, pharmacological and molecular techniques (Teramoto *et al*., 2001). Thus, under the present experimental conditions, we suggest that the inward Ba^{2+} currents studied in pig urethral myocytes flow mainly through L-type Ca^{2+} channels (i.e. $Ca_V1.2$), which are sensitive to nifedipine and calciseptine (Teramoto *et al*., 1996, 2001).

Figure 2 Effects of mefenamic acid (300 μM) on voltage-dependent Ba^{2+} inward currents at a holding membrane potential of −50 mV in pig urethra. The pipette solution was Cs^+-TEA^+ solution containing 5 mM EGTA and the bath solution was 10 mM Ba^{2+} containing 135 mM TEA^+. (a) (i) Original current traces before (control) and after application of 300 μM mefenamic acid at the indicated pulse potentials. (ii) Inward Ba^{2+} current from (i) scaled to match their peak amplitudes and superimposed. (b) Current–voltage relationships obtained in the absence (control) or presence of 300 μM mefenamic acid. The current amplitude was measured as the peak amplitude of the Ba^{2+} inward current in each condition. The lines were drawn by eye. (c) Relationship between the test potential and relative value of the Ba^{2+} inward currents by 300 μM mefenamic acid, expressed as a fraction of the peak amplitude of the Ba^{2+} inward current evoked by various amplitude of the depolarizing pulse in the absence of mefenamic acid. Each column indicates the mean of four observations with + s.d. shown by vertical lines. *Significantly different from control (ANOVA, $P<0.05$).

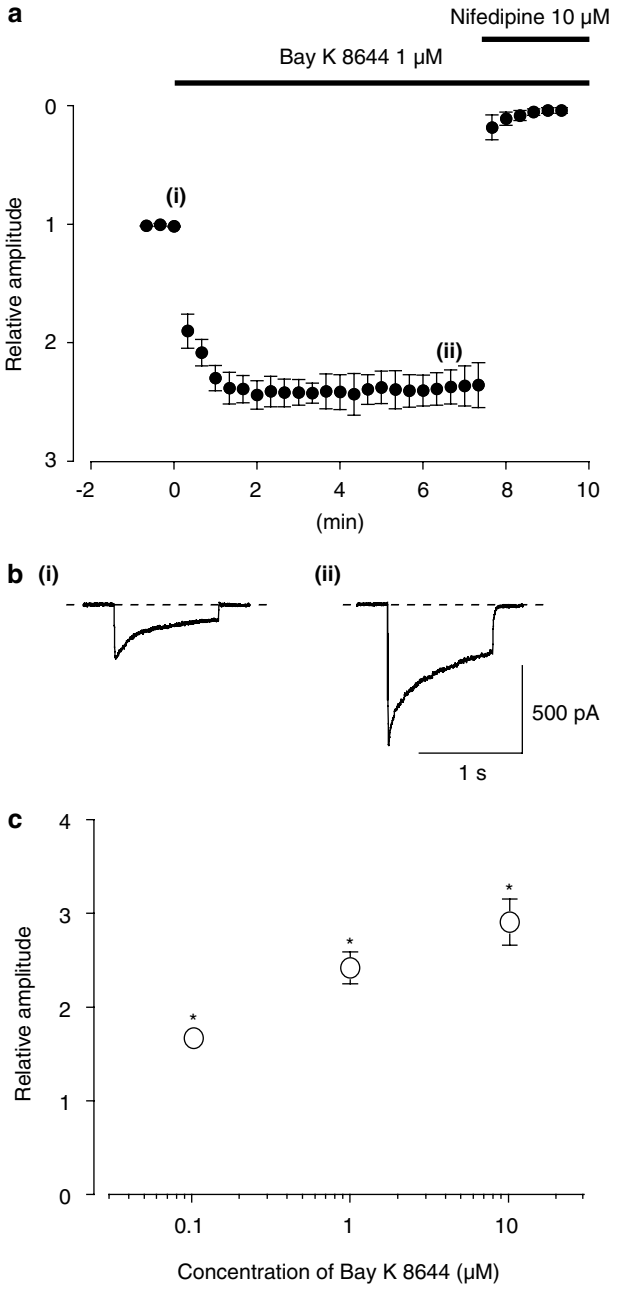


Figure 3 Effects of Bay K8644 (1 μM) on voltage-dependent Ba^{2+} currents using conventional whole-cell recording from an isolated pig urethral myocyte. (a) The time course of activation of the peak amplitude of voltage-dependent Ba^{2+} currents by Bay K8644 (1 μM) is shown. Inward currents were elicited by voltage steps (1 s duration) to +10 mV from a holding potential of −50 mV every 20 s. Time 0 indicates the time when Bay K8644 was applied. The activtion produced by Bay K8644 was suppressed by nifedipine 10 μM. Each symbol indicates the mean of six observations with ± s.d. shown by vertical lines. Some of the s.d. bars are less than the size of the symbol. (b) The three traces show inward currents, elicited by voltage steps in control solution (i), after the application of Bay K8644 (ii). (c) Relationships between relative activation of the peak amplitude of Ba^{2+} current and the concentration of Bay K8644 at a holding potential of −50 mV. The peak amplitude of the Ba^{2+} current elicited by a step pulse to +10 mV from −50 mV just before application of Bay K8644 was normalized as one. Each symbol indicates the mean of 6–8 observation with ± s.d. shown by vertical lines. Some of the s.d. bars are less than the size of the symbol. *Significantly different from control (ANOVA, $P<0.05$).

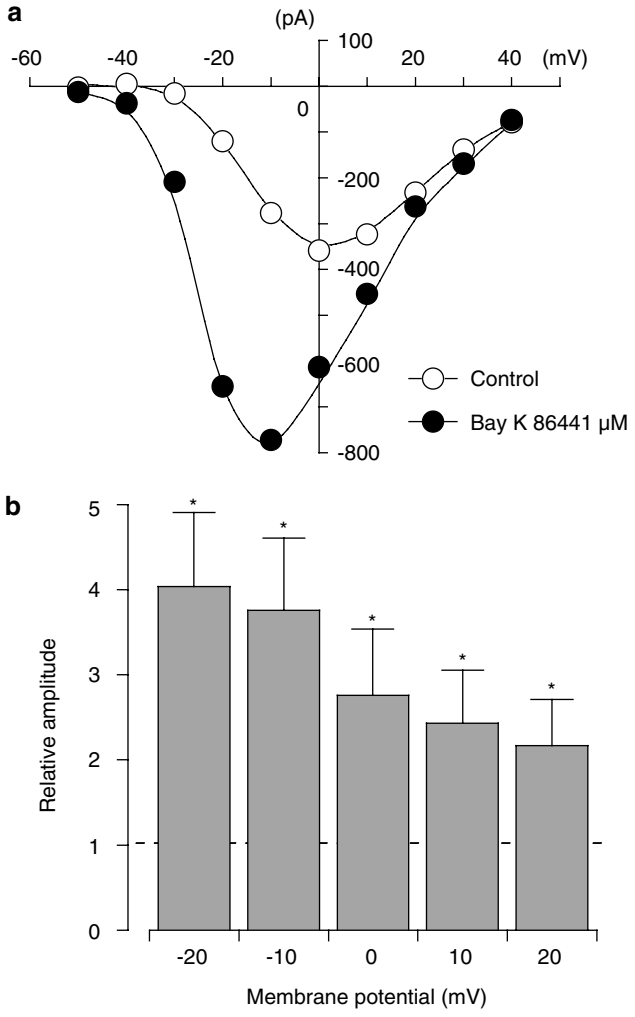


Figure 4 Effects of Bay K8644 (1 μM) on voltage-dependent Ba^{2+} inward currents at a holding membrane potential of −50 mV in pig urethra. The pipette solution was Cs^{+}-TEA^{+} solution containing 5 mM EGTA and the bath solution was 10 mM Ba^{2+} containing 135 mM TEA^{+}. (a) Current–voltage relationships obtained in the absence (control) or presence of Bay K8644 (1 μM). The current amplitude was measured as the peak amplitude of the Ba^{2+} inward current in each condition. The lines were drawn by eye. (b) Relationship between the test potential and relative value of the Ba^{2+} inward currents activated by Bay K8644 (1 μM), expressed as a fraction of the peak amplitude of the Ba^{2+} inward current evoked by various amplitude of the depolarizing pulse in the absence of Bay K8644. Each column indicates the mean of six observations with ± s.d. shown by vertical lines. *Significantly different from control (ANOVA, $P<0.05$).

Activating mechanisms of voltage-dependent Ba^{2+} currents by mefenamic acid

In the present experiments, we have demonstrated that mefenamic acid and Bay K 8644 enhanced the peak amplitude of voltage-dependent Ba^{2+} currents without changing the reversal potential for Ba^{2+} (E_{rev}) and the threshold for voltage-dependent Ba^{2+} currents. However, there were several differences between mefenamic acid and Bay K 8644 regarding the enhancement of voltage-dependent Ba^{2+} currents. (1) Although Bay K 8644 shifted the maximum of the current–voltage relationship towards more negative potentials, mefenamic acid did not change the position of the maximum.

(2) Bay K 8644 enhanced the peak amplitude of voltage-dependent Ba^{2+} currents in a voltage-dependent manner. However, mefenamic acid increased the peak amplitude of voltage-dependent Ba^{2+} currents with little voltage-dependence. (3) Bay K 8644 caused little shift of the voltage-dependence of the inactivation curve to more negative potentials. In contrast, the inactivation curve was shifted to the more positive potentials in the presence of mefenamic acid. (4) Bay K 8644 shifted the activation curve to more negative potentials, whereas mefenamic acid caused no significant shift of the activation curve. Given this, we suggest that mefenamic acid increased voltage-dependent Ba^{2+} currents through the activation of L-type Ca^{2+} channels with different kinetics from those of Bay K 8644 in pig urethral myocytes.

We suggest that the effects of Bay K 8644 on the current–voltage relationship might be the consequence of the hyperpolarizing shift in the activation curve. Bay K 8644 shifted the activation curves to more negative potentials and caused a significant increase in the slope of activation, which reflects a yield of more current for a given potential. These results indicate that Bay K 8644 may alter the voltage-sensitivity of the channels.

Although mefenamic acid caused little shift of the voltage-dependence of the activation curve, mefenamic acid shifted voltage-dependence of the inactivation curve to more positive potentials, increasing the area of window-currents of voltage-dependent Ca^{2+} channels (reviewed by Davila, 1999). Thus, the kinetic mechanisms of mefenamic acid for the activation of voltage-dependent Ca^{2+} currents are likely to be quite different from those of Bay K 8644.

Newly synthesized compounds for voltage-dependent Ca^{2+} channels

Several newly synthesized compounds, some dihydropyridine derivatives such as Bay K 8644 (Hess *et al*., 1984), CGP 28392 (Kokubun & Reuter, 1984), RS 30026 (Patmore *et al*., 1990), (+) 1,4-dihydro-2, 6-dimethyl-5-nitro-4-(benzofuran-5-yl) pyridine-3-carboxylate (Visentin *et al*., 1999) and a benzoyl-pyrrole compound (FPL 64176; Rampe & Lacerda, 1991) have been shown to possess predominantly agonistic activity of L-type Ca^{2+} channels. In the present experiments, we have been able to demonstrate that mefenamic acid, one of the most

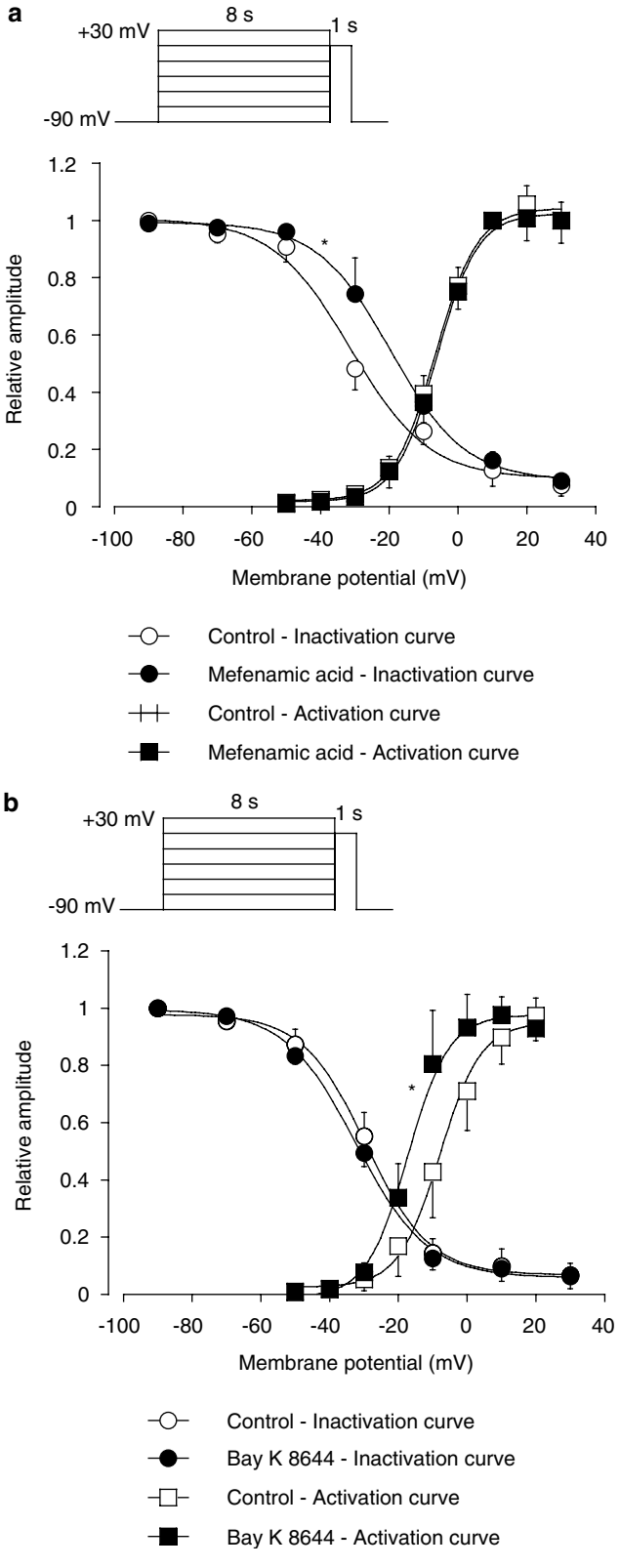


Figure 5 Effects of mefenamic acid and Bay K8644 on the voltage-dependent activation and inactivation of the Ba^{2+} inward currents in pig urethra. Whole-cell recording, pipette solution Cs^{+}-TEA^{+} solution containing 5 mM EGTA and the bath solution 10 mM Ba^{2+} containing 135 mM TEA^{+}. Steady-state inactivation curves, obtained in the absence (control) and presence of mefenamic acid and Bay K8644, were fitted to the Boltzmann equation. Peak current values were used. The steady-state inactivation curve was obtained using the double-pulse protocol (see Methods). The current measured during the test pulse is plotted against membrane potential and expressed as relative amplitude and in % of control. Activation curves were obtained from the current–voltage relationships of Figures 2 and 4, fitting to the Boltzmann equation (see Methods). (a) The steady-state inactivation curves in the absence or presence of mefenamic acid were drawn using the following values: (control), $I_{max}=1$, $V_{half}=-31$, $k=11$ and $C=0.1$; (mefenamic acid, 300 μM), $I_{max}=1$, $V_{half}=-20$, $k=11$ and $C=0.09$. Each symbol indicates the mean of four observations with $\pm$s.d. shown by vertical lines. Some of the s.d. bars are less than the size of the symbol. (b) The steady-state inactivation curves in the absence or presence of Bay K8644 were drawn using the following values: (control), $I_{max}=0.91$, $V_{half}=-29$, $k=9$ and $C=0.07$; (Bay K8644, 1 μM), $I_{max}=1.8$, $V_{half}=-32$, $k=11$ and $C=0.11$. Each symbol indicates the mean of four observations with $\pm$s.d. shown by vertical lines. Some of the s.d. bars are less than the size of the symbol. *Two curves in the absence (control) and presence of drug (mefenamic acid or Bay K8644) were significantly different (ANOVA, $P<0.05$).

potent fenamates, also increased the activity of L-type Ca^{2+} channels although its chemical structure is very different. Surprisingly, higher concentrations of mefenamic acid ($100\,\mu M \geqslant$) were needed to cause an activation of Ca^{2+} channel activity. We have recently demonstrated the multiple actions of mefenamic acid on K^+ currents in pig urethral myocytes in a similar concentration range (see Introdution, Teramoto *et al*., 2003). Thus, it is difficult to define that mefenamic acid is a selective activator of L-type Ca^{2+} channels due to its multiple actions. However, we suggest that this might provide useful information for the synthesis of novel types of L-type Ca^{2+} channel activators and that mefenamic acid may serve as a useful prototype for designing selective activating compounds for L-type Ca^{2+} channels.

In conclusion, we have been able to demonstrate that mefenamic acid activates L-type Ca^{2+} channels with a different mechanism from that of Bay K 8644 in pig urethral myocytes.

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