The effects of flufenamic acid on spontaneous activity of smooth muscle tissue isolated from the guinea-pig stomach antrum

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Abstract

The effects of flufenamic acid were investigated on slow waves, follower potentials and pacemaker potentials recorded respectively from circular smooth muscle cells, longitudinal smooth muscle cells and interstitial cells of Cajal distributed in the myenteric layers (ICC-MY) of the guinea-pig stomach antrum. Flufenamic acid (>10⁻⁵ M) inhibited the amplitude and rate of rise of the upstroke phase of the slow waves, with no marked alteration in their frequency of occurrence. The inhibitory actions of flufenamic acid appeared to be mainly on slow potentials recorded from circular smooth muscle cells, but not on follower or pacemaker potentials. After abolishing spontaneous slow potentials with flufenamic acid, depolarizing current stimuli could evoke slow potentials with an amplitude that was much smaller than in the absence of flufenamic acid, with no significant alteration to the input resistance of the membrane. The time elapsed for the generation of the 2nd component of the slow waves or the slow potentials evoked during depolarizing current pulse stimulation was increased by flufenamic acid. The rate of rise of unitary potentials, but not the frequency of occurrence, was inhibited by flufenamic acid. These results indicate that the inhibitory actions of flufenamic acid appear to be mainly on the circular muscle layer including the interstitial cells of Cajal distributed within the muscle bundles (ICC-IM). Nifedipine-sensitive spike potentials were not inhibited by flufenamic acid. It is concluded that the selective inhibition of the 2nd component of slow waves by flufenamic acid may be mainly due to the inhibition of ion channels, possibly Ca²⁺-sensitive Cl⁻-channels, activated during generation of slow potentials in the ICC-IM distributed in the circular muscle layer.

Key words: flufenamic acid, gastric muscle, pacemaker, slow wave, Ca-sensitive Cl⁻-channel

Introduction

Gastric smooth muscle is spontaneously active with rhythmic generation of slow waves or action potentials or both (Tomita, 1981), with interstitial cells distributed in the myenteric
region (ICC-MY) providing the pacemaker activity for these rhythmic activities (Sanders, 1996; Huizinga et al., 1997; Sanders et al., 1999; Suzuki, 2000; Hirst and Ward, 2003; Takaki, 2003). Gap junctions formed between ICC-MY with surrounding ICC-MY and smooth muscle cells (Komuro et al., 1999) enable pacemaker potentials generated in the ICC-MY to propagate to smooth muscle cells in an electrotonic manner (Dickens et al., 1999; Cousins et al., 2000; Hirst and Ward, 2003). In gastric muscle of the guinea-pig, the pacemaker potentials are composed of two components, a primary component which forms the initial transient phase followed by a secondary plateau component (Hirst and Edwards, 2001; Kito et al., 2002; Kito and Suzuki, 2003b). The primary component may be formed by Ca\(^{2+}\)-permeable ion channels which are sensitive to voltage, since this component is inhibited in low Ca\(^{2+}\) solution or during depolarization of the membrane (Hirst and Edwards, 2001; Kito et al., 2002; Kito and Suzuki, 2003b). The plateau component is inhibited in low Cl\(^{-}\) solution, in the presence of chemicals which inhibit Ca\(^{2+}\)-activated Cl\(^{-}\)-channels (niflumic acid, DIDS, 9-AC) or chemicals known to chelate intracellular Ca\(^{2+}\) (BAPTA, MAPTA), suggesting that this component is formed by activation of Ca\(^{2+}\)-sensitive Cl\(^{-}\)-channels (Hirst et al., 2002; Kito et al., 2002; Kito and Suzuki, 2003b; Hirst and Ward, 2003).

Isolated circular muscle tissue of the guinea-pig stomach antrum is also spontaneously active, with periodic generation of regenerative slow potentials (Suzuki and Hirst, 1999). The slow potentials may be formed by the summation of unitary potentials generated in interstitial cells distributed within muscle bundles (ICC-IM) (Edwards et al., 1999; Dickens et al., 2001; Edwards and Hirst, 2005). The pharmacological properties of slow potentials differ from those of slow waves in that slow potentials but not slow waves are inhibited by low concentrations (0.5–1 mM) of caffeine (Suzuki and Hirst, 1999; Nose et al., 2000; Fukuta et al., 2002). Slow potentials are not inhibited by nifedipine, but can be inhibited by chemicals known to interfere with Ca\(^{2+}\)-activated Cl\(^{-}\)-currents (Hirst et al., 2002), suggesting that unitary potentials are formed by activation of Ca\(^{2+}\)-sensitive Cl\(^{-}\)-channel currents.

Flufenamnic acid is a known inhibitor of cyclooxygenase (Flower, 1974). In addition, this chemical modulates many types of ion channels distributed in different types of mammalian tissues including smooth muscle, such as activation of K-channels (Farrugia et al., 1993; Xu et al., 1994; Greenwood and Large, 1995; Shimamura et al., 2002) and inhibition of L-type Ca\(^{2+}\)-channels (Doughty et al., 1998), Ca\(^{2+}\)-sensitive Cl\(^{-}\)-channels (Greenwood and Large, 1995), non-selective cation channels (Chen et al., 1993) or Ca\(^{2+}\)-permeable non-selective cation channels (Hill et al., 2004). Thus, flufenamnic acid has wide inhibitory actions on a range of ion channels in a rather non-specific way.

It was of interest to investigate the effects of flufenamic acid on the spontaneous activity of gastric smooth muscle tissue, since this chemical may inhibit the generation of slow waves due to a non-selective inhibition of ion channels. Experiments were carried out to investigate the effects of flufenamic acid on slow waves generated in smooth muscle isolated from the guinea-pig stomach antrum. The results indicate that in concentrations >10\(^{-5}\) M, flufenamic acid reduces the amplitude of slow waves, mainly by inhibiting the slow potential component generated in the circular muscle. The inhibition of follower or pacemaker potentials by flufenamic acid was very weak, and rather non-selective inhibitory actions appeared only when
relatively high concentrations (>10^{-4} M) were applied.

**Methods**

**Preparations**

Male albino guinea-pigs, weighing 200–300 g, were anesthetized with fluoromethyl 2,2,2-trifluoro-1-(trifluoromethyl) ethyl ether (sevoflurane, Maruishi Pharm., Osaka, Japan), and exsanguinated by decapitation. All animals were treated ethically according to the guiding principles for the care and use of animals in the field of physiological sciences, approved by The Physiological Society of Japan. The stomach was excised, and opened by cutting along the small curvature in Krebs solution. The mucosal layers were removed by cutting with fine scissors, and smooth muscle tissue was isolated from the antrum region.

A segment of tissue (about 1.5 mm width and 3 mm long) was dissected, and three types of tissue preparation were made. The intact muscle preparation contained single bundles of circular muscle (100 μm wide × 200 μm long) which were attached to the underlying longitudinal muscle and myenteric plexus regions. This was pinned out mucosal-side uppermost onto a silicone rubber plate fixed at the bottom of the organ bath (10 mm wide, 20 mm long, 1 mm deep). The longitudinal muscle preparation (300 μm × 500 μm) was prepared by removing the circular muscle layer, and also pinning it mucosal-side uppermost onto the silicone rubber plate. The circular muscle preparation was made by removing the longitudinal muscle layer with the attached myenteric plexus. The single circular smooth muscle bundle preparation (150–200 μm long) was isolated, and pinned out serosal-side uppermost onto the silicone rubber plate. These muscle preparations were superfused with warmed (36°C) and oxygenated Krebs solution, at a constant flow rate of about 2 ml/min. Most of the experiments were carried out in the presence of 1 μM nifedipine throughout, so as to minimize muscle movements.

Conventional microelectrode techniques were used to record the electrical activity of smooth muscle cells, using glass capillary microelectrodes (outer diameter, 1.2 mm, inner diameter 0.6 mm; Hilgenberg, Germany) filled with 0.5 M KCl (tip resistances ranging between 150 and 300 MΩ). In circular smooth muscle preparations, responses were recorded from two cells simultaneously using two electrodes; one electrode for the current stimulation and another for recording electrical responses of smooth muscle cells. Electrical responses were displayed on a cathode-ray oscilloscope (SS-7602, Iwatsu, Osaka, Japan) and also stored on a personal computer for later analysis, through a high input impedance amplifier (Axoclamp-2B, Axon Instruments, Inc., Foster City, California, U.S.A.).

The Krebs solution had the following ionic composition (mM): Na⁺ 137.4, K⁺ 5.9, Ca²⁺ 2.5, Mg²⁺ 1.2, HCO₃⁻ 15.5, H₂PO₄⁻ 1.2, Cl⁻ 134, and glucose 11.5. The solutions were aerated with O₂ containing 5% CO₂, and the pH of the solutions was maintained at 7.2–7.3.

Drugs used were nifedipine and flufenamic acid purchased from Sigma, San Diego, California, U.S.A. Nifedipine or flufenamic acid was dissolved in dimethyl sulphoxide (DMSO) to make stock solutions, and they were added to Krebs solution to make the desired concentrations, just prior to the use. The final concentration of the solvent in Krebs solution did not exceed 1/1000. Addition of these chemicals to Krebs solution did not alter the pH of the solution.
Fig. 1. Effects of flufenamic acid on slow waves recorded from gastric muscle. Slow waves were recorded from circular muscle cells of intact muscle preparations, in the absence (A) and presence of flufenamic acid (B, 10^{-6} M; C, 10^{-5} M; D, 10^{-4} M). Records A-D were obtained from the same cell.

Experimental values were expressed as the mean value ± standard deviation (SD). Statistical significance was tested using the Student's t-test, and probabilities of less than 5% (P<0.05) were considered to be significant.

Results

Effects of flufenamic acid on slow waves

In intact preparations of the stomach antrum, experiments were carried out to observe the effects of different concentrations of flufenamic acid (10^{-6} M–10^{-4} M) on slow waves. In the absence of flufenamic acid, the circular muscle produced slow waves periodically with an amplitude and frequency of 28.1 ± 5.5 mV and 2.9 ± 0.8 cycles per min (cpm), respectively (n=18). With application of flufenamic acid, the amplitude of the slow waves was reduced in concentrations above 10^{-5} M, but with no marked alteration to the frequency (Fig. 1).

The effects of flufenamic acid on the parameters of slow waves (amplitude, duration, rate of rise of the upstroke phase and frequency) were quantified. Slow waves are comprised of two components, referred to as the 1st and 2nd components by Tomita (1981). The first component is an electrotonic potential consisting of pacemaker potentials, while the second component consists of slow potentials generated in the circular muscle (Hirst and Ward, 2003). The effects of flufenamic acid on the elapsed time between the generation of the 2nd component following the onset of the 1st component (latency) were also quantified. In concentrations ranging between 10^{-6} and 3×10^{-5} M, flufenamic acid did not alter the resting membrane potential, but at
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10^{-4} M this chemical depolarized the membrane by 2-7 mV (mean, 4.2 ± 1.2 mV, n=5; Fig. 2A). The amplitudes of the 1st component and peak (i.e., the sum of 1st and 2nd components) were measured in the presence of flufenamic acid, and the results indicated that the peak amplitude was decreased by >3 x 10^{-6} M flufenamic acid and the 1st component by 10^{-4} M flufenamic acid (Fig. 2B). The duration was measured as the time elapsed between the onset and offset at the base of each of the 1st and 2nd components of the slow waves. Flufenamic acid (>3 x 10^{-5} M) reduced the duration of the 2nd component of slow waves with no alteration to the 1st component (Fig. 2C). The latency for the generation of the 2nd component of the slow waves was increased significantly by 10^{-4} M flufenamic acid, but not by lower concentrations of flufenamic acid (Fig. 2D). The maximum values of the rate of rise of the upstroke of the slow waves (dV/dt) were measured for the 1st and 2nd components. The dV/dt for the 1st component was only inhibited by 10^{-4} M flufenamic acid while that for the 2nd component was inhibited by >10^{-3} M flufenamic acid (Fig. 2E). The frequency of slow waves remained unaltered by flufenamic acid, in concentrations of up to 10^{-4} M (Fig. 2F). These results indicated that flufenamic acid mainly inhibited the 2nd component of the slow waves.

Attempts were made to compare the effects of flufenamic acid on the amplitude of slow waves, follower potentials and pacemaker potentials, recorded from the circular smooth muscle of the intact muscle preparations and the longitudinal smooth muscle and ICC-MY of the

Fig. 2. Effects of flufenamic acid on the parameters of slow waves recorded from circular muscle cells of intact muscle preparations. Slow waves were recorded in the absence and presence of different concentrations of flufenamic acid (10^{-6}-10^{-4} M). The resting membrane potential (A), amplitude (B), duration (C), latency before the 2nd component (D), the rate of rise of the upstroke phase (dV/dt, E) and the frequency (F) of the slow waves were measured. The amplitude, duration and dV/dt were measured for the 1st and 2nd components of the slow waves (●, 1st component; ○, 2nd component). Mean ± S.D. (n=6-12). *, significant from control.
Effects of flufenamic acid on slow waves, follower potentials and pacemaker potentials recorded from gastric smooth muscle cells. Slow waves (A), follower potentials (B) and pacemaker potentials (C) were recorded from circular smooth muscle cells of intact muscle preparations and from longitudinal smooth muscle cells and ICC-MY of longitudinal muscle preparations, respectively, in the absence (a1, b1, c1) and presence of $10^{-4}$ M flufenamic acid (a2, b2, c2). Each pair of responses was recorded from the same cell in different preparations. D. graphic representation of the effects of different concentrations of flufenamic acid on the amplitude of slow waves, follower potentials and pacemaker potentials. The amplitude was shown as relative to that before application of flufenamic acid. Mean ± S.D. (n=7). *, significant from control responses generated in the absence of flufenamic acid.

longitudinal muscle preparations, respectively. As exemplified in Fig. 3, the inhibitory actions were marked on slow waves compared to those on follower potentials and pacemaker potentials. The quantified graph indicated that slow waves were inhibited by $>10^{-5}$ M flufenamic acid whereas the follower potentials and the pacemaker potentials were inhibited by about 10% at $10^{-4}$ M flufenamic acid (Fig. 3D).

Effects of flufenamic acid on slow potentials

Experiments were carried out to examine the effects of flufenamic acid on the slow potentials generated in circular muscle preparations. One of the typical examples of the effects of increasing concentrations of flufenamic acid on slow potentials is shown in Fig. 4. In this preparation, slow potentials were abolished by $10^{-6}$ M flufenamic acid, with no marked alteration of the resting membrane potential (control, $-65.6 ± 2.3$ mV, n=12; in $10^{-5}$ M flufenamic acid, $-65.3 ± 2.5$ mV; n=18; P>0.05). Lower concentrations of flufenamic acid ($10^{-6} - 3 × 10^{-6}$ M) did not alter either the amplitude or the frequency of slow potentials. This was also the case in 5 other preparations, while in 1 preparation $10^{-6}$ M flufenamic acid abolished spontaneous slow potentials whereas in 2 other preparations $3 × 10^{-5}$ M flufenamic acid was required to abolish the slow potentials. Unitary potentials were still generated, after the generation of spontaneous slow potentials had been inhibited by flufenamic acid (Fig. 4C).
Fig. 4. Effects of flufenamic acid on slow potentials generated in circular muscle preparations. In a small segment of a circular muscle preparation (about 100 μm x 250 μm), electrical responses were recorded from a smooth muscle cell, in the absence (A) and presence of flufenamic acid (B, 3 x 10^-5 M; C, 10^-5 M). All responses were obtained from the same cell. The relationship between concentration of flufenamic acid and amplitude (D) and frequency (E) of spontaneously generated slow potentials. Mean ± S.D. (n=5–6). *, significant inhibition (P<0.05).

The properties of the electrical responses evoked by hyperpolarizing and depolarizing current stimuli were investigated in circular muscle preparations, after the generation of spontaneous slow potentials had been abolished by 10^-5 M flufenamic acid. In these experiments, electrical responses were recorded from two different cells simultaneously. The activities of any given pair of cells were synchronized, either in the absence or presence of flufenamic acid, indicating that both cells were electrically coupled, as observed previously (Suzuki and Hirst, 1999). In the absence of flufenamic acid, a hyperpolarizing current stimulus (0.5–2.5 nA intensity, 5 s duration) produced an electrotonic potential with sustained hyperpolarization, and a slow potential was evoked, followed by the termination of the electrotonic potential (Fig. 5A). The amplitude of hyperpolarization increased linearly with increasing current intensity (Fig. 5E), while the input resistance calculated from the relationship ranged between 1 and 3 MΩ (mean, 1.9 ± 0.1 MΩ, n=5). In the absence of slow potentials as a result of the application of 10^-5 M flufenamic acid, stimulation of the muscle with a hyperpolarizing current produced an electrotonic potential similar to that produced before application of flufenamic acid, but with no associated generation of slow potentials after cessation of the current stimulus (Fig. 5C). The relationship between the amplitude of the electrotonic potentials and the intensity of the stimulating current indicated that it was identical to that observed in the absence of flufenamic acid (Fig. 5E). The input resistance calculated from the relationship was 1.8 ± 0.1 MΩ (n=5), a value that was not significantly different from that observed in the absence of flufenamic acid (P>0.05). These results indicated that the
biophysical properties of the membrane were not altered during the inhibition of the slow potentials by flufenamic acid.

In the absence of flufenamic acid, depolarizing current stimuli evoked slow potentials with amplitudes that were similar at any given intensity of >0.5 nA (Fig. 5B), and which were not significantly different to those generated spontaneously (*P>0.05; Fig. 5F). In the presence of 10^{-5} M flufenamic acid, depolarization of the membrane again evoked slow potentials (Fig. 5D). The quantified data indicated that the threshold intensity of the current stimuli required to evoke slow potentials was increased to 1.0 nA and the amplitude was smaller than in the absence of flufenamic acid, at any given intensity of stimulus (Fig. 5F).

The latency for the generation of slow potentials in response to depolarizing current stimuli was a function of the intensity of the current, and it decayed to a stable value of about 1 s by increasing the intensity of the stimulus, as reported previously (Suzuki and Hirst, 1999). In the preparation shown in Fig. 5, the latency was about 1 s at a stimulus strength of >1.5 nA. In the presence of flufenamic acid, the latency was significantly increased at any given intensity of
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Fig. 6. Effects of flufenamic acid on unitary potentials. In circular muscle preparation, unitary potentials were recorded in the absence (A) and presence of $10^{-5}$ M flufenamic acid (B). C, the rate of rise of the upstroke phase of unitary potentials with an amplitude $>2$ mV (Mean ± S.D., n=55). *, significant from control. D, frequency power spectrum of the unitary potentials recorded in the absence (●) and presence of $10^{-5}$ M flufenamic acid (○).

current and reached a stable value of about 2 s at 2 nA intensity (Fig. 5G). These results indicated that flufenamic acid inhibited the generation of slow potentials by elevating the threshold for excitation, with no alteration to the input resistance of the smooth muscle membrane.

The effects of flufenamic acid on unitary potentials were examined in circular smooth muscle preparations, since summation of these potentials is considered to form slow potentials (Edwards et al., 1999). In all preparations examined (n=18), unitary potentials were generated in the absence or presence of $10^{-5}$ M flufenamic acid (Fig. 6A and B). The frequency analysis of unitary potentials indicated that the power spectrum was not altered by flufenamic acid (Fig. 6D), indicating that the occurrence of unitary potentials was not changed by this chemical. The $dV/dt$ of the upstroke phase of unitary potentials was measured only for those with an amplitude of $>2$ mV, and the results indicated that the speed of the upstroke was significantly reduced by flufenamic acid (Fig. 6C).

Effects of flufenamic acid on action potentials

The effects of flufenamic acid on the action potentials generated on the slow potentials elicited by depolarizing stimuli were observed in circular muscle preparations. In the absence of nifedipine, depolarizing current stimuli elicited slow potentials with a repetitive generation of spike potentials (Fig. 7A). After inhibiting the generation of spontaneous slow potentials by $10^{-5}$ M flufenamic acid, depolarizing stimuli again elicited spike potentials superimposed on a slow
Fig. 7. Effects of flufenamic acid on spike potentials. In a circular muscle preparation, electrical responses were recorded from two cells simultaneously in the absence of nifedipine. A depolarizing current pulse (5 s duration, 2 nA intensity) was applied to one electrode and the responses generated in cells recorded from the 2nd electrode, in the absence (A) and presence of $10^{-5}$ M flufenamic acid (B). A and B were recorded from the same preparation, but different cells. In each set of traces, upper and lower traces indicate the changes in membrane potential and the current applied to the stimulating electrode, respectively.

potential, but with an increased latency for the generation of the slow potentials (Fig. 7B). The dV/dt of the spike potentials was $740 \pm 133$ mV/s ($n=45$), and this was not significantly changed by flufenamic acid ($787 \pm 155$ mV/s, $n=55$; $P>0.05$). These results indicate that flufenamic acid does not alter the properties of nifedipine-sensitive channels activated in circular smooth muscle of the stomach antrum.

Discussion

The present experiments showed that in smooth muscle isolated from the guinea-pig stomach antrum, flufenamic acid reduced the amplitude of slow waves with no marked alteration in their frequency. Slow waves are comprised of two components, the 1st and 2nd components (Tomita, 1981), and each is formed respectively by a propagated electrotonic potential of pacemaker potentials and of regenerative slow potentials generated in the circular muscle (Cousins et al., 2003; Hirst and Ward, 2003; Edwards and Hirst, 2005). The inhibition of slow waves by flufenamic acid occurred mainly on the 2nd component, which suggests that it was the activity of circular smooth muscle cells that was mainly inhibited. This is supported by the difference in the inhibitory effects of flufenamic acid between slow potentials recorded from circular muscle preparations and the pacemaker or follower potentials recorded directly from ICC-MY or longitudinal smooth muscle cells, respectively. The threshold concentration of flufenamic acid required to abolish the slow potentials was $10^{-5}$ M, while pacemaker potentials
or follower potentials were inhibited only partially by $10^{-4}$ M. Depolarization by high concentrations of flufenamic acid (>10^{-4} M) must also be taken into account as one of the factors which could reduce the amplitude of these potentials. The fact that flufenamic acid selectively inhibited the amplitude, but not the frequency and duration of slow waves, also indicates that this chemical mainly inhibits slow potential components generated in circular muscle cells. The resting membrane potential of smooth muscle cells was not markedly altered by these effective concentrations of flufenamic acid, suggesting that the ionic conductance of the membrane was not changed. This was confirmed by the absence of significant change in the input resistance of the membrane during the inhibition of slow potentials with flufenamic acid.

Flufenamic acid modulates many types of ion channels, such as its activation of voltage-dependent K^-channels distributed in vascular smooth muscle (Farrugia et al., 1993; Xu et al., 1994; Greenwood and Large, 1995; Shimamura et al., 2002), as well as its inhibition of voltage-sensitive L-type Ca^{2+}-channels distributed in smooth muscle cells of both the rabbit basilar artery (Doughty et al., 1998) and the rat carotid artery (Shimamura et al., 1998), the Ca^{2+}-sensitive Cl^-channels distributed in the rabbit portal vein (Greenwood and Large, 1995), the non-selective cation channels distributed in the guinea-pig ileal smooth muscle (Chen et al., 1993) or the Ca^{2+}-permeable non-selective cation channels (TRPM2 channels) expressed in cultured human embryonic kidney cells (Hill et al., 2004). The present experiments have indicated that flufenamic acid did not inhibit L-type Ca^{2+}-channels in gastric smooth muscle of the guinea-pig, as nifedipine-sensitive spike potentials were not altered by this chemical. Slow potentials generated in the circular muscle preparations may be formed by the summation of unitary potentials which are propagated from intramuscular interstitial cells (ICC-IM) in an electrotonic manner (Edwards et al., 1999; Dickens et al., 2001; Edwards and Hirst, 2005). The frequency analysis of these unitary potentials revealed that the occurrence of unitary potentials was not altered during the inhibition of slow potentials by flufenamic acid, suggesting that the inhibitory actions appeared on the summation mechanism of unitary potentials but not on the generating mechanism. The inhibitory actions of flufenamic acid appeared to be mainly on the speed of the upstroke phase of the unitary potentials, which also supported the concept that the inhibition occurs mainly on ionic channels contributing to the formation of the upstroke phase of unitary potentials. In circular muscle preparations, slow potentials and unitary potentials may be produced by activation of Ca^{2+}-sensitive Cl^-channels (Hirst et al., 2002), and the strong inhibition by flufenamic acid of the slow potentials supports this concept, since this chemical is known to inhibit these channels (Greenwood and Large, 1995).

The Ca^{2+}-sensitive Cl-conductance may also be playing a central role in the plateau component formation of the pacemaker potential generated in ICC-MY (Kito et al., 2002; Kito and Suzuki, 2003a). The present experiments revealed that the inhibitory actions of flufenamic acid on pacemaker potentials were very weak, and that their duration was not altered when the amplitude was reduced by about 10% with $10^{-4}$ M flufenamic acid. These results indicate a possibility that the properties of Ca^{2+}-sensitive Cl^-channels activated during formation of the plateau potential are not identical to those activated during generation of slow potentials or unitary potentials. The pacemaker potentials and unitary potentials (or slow potentials) are generated in ICC-MY and ICC-IM, respectively, and it is speculated that the ionic mechanism
responsible for the generation of these potentials may not be identical, although chemicals such as DIDS and niflumic acid could not differentiate between them.

The initial phase of the pacemaker potentials is sensitive to Ni²⁺ (Tomita et al., 1998; Hirst and Edwards, 2001; Kito and Suzuki, 2003b), and is also inhibited by the reduction of [Ca²⁺]₀ concentrations or by depolarization of the membrane (Kito et al., 2002; Kito and Suzuki, 2003b), suggesting that it is formed by the activation of voltage-sensitive, but nifedipine-insensitive, Ca²⁺-permeable channels. In cultured ICC-MY isolated from the mouse small intestine, the ionic mechanism for generating the pacemaker currents is equivocal, and the possible involvement of Ca²⁺-sensitive Cl⁻-channels (Tokutomi et al., 1995), non-selective cation channels (Thomsen et al., 1998) or Ca²⁺-inhibited non-selective cation channels (Koh et al., 2002) is proposed. Recent reports indicate that in cultured ICC-MY of the mouse intestine the pacemaker current is inhibited by flufenamic acid, as well as by Ni²⁺, Mn²⁺, Cd²⁺ or Co²⁺: the concentrations of flufenamic acid inhibiting the pacemaker currents are comparable to those effective for inhibiting slow potentials in the guinea-pig stomach (equal to 10⁻⁵ M; Jun et al., 2004). However in intact tissues isolated from the mouse intestine, the initial component of the pacemaker potential recorded from ICC-MY is inhibited by mibebradil, a non-selective inhibitor of voltage-dependent Ca²⁺ channels (Kito et al., 2005), while the plateau component is inhibited by chemicals known to inhibit the Ca²⁺-sensitive Cl⁻-channels (Kito and Suzuki, 2003a). These data suggest that the properties of pacemaker potentials recorded from cultured cells are not always identical to those obtained from intact tissues. The present experiments showed that in intact tissues, flufenamic acid did not inhibit the dV/dt of the pacemaker potential component recorded from gastric ICC-MY. Thus, although the inhibitory actions of flufenamic acid are reportedly occurring on many types of ion channels, there appears to be a rather selective inhibition of Ca²⁺-sensitive Cl⁻-channels in the case of the gastric muscle of the guinea-pig stomach antrum.

The present experiments have demonstrated that flufenamic acid delayed the generation of slow potentials during depolarization of the membrane. This was comparable to the increase in the latency of the 2nd component of the slow waves by flufenamic acid. The latency for generating the slow potential in response to depolarization is a function of the amplitude of depolarization, and decays with increasing stimulus intensity to reach a stable value of about 1 s (Suzuki and Hirst, 1999). This delay may be the result of the formation of unidentified mediators required for the generation of slow potentials during depolarization, and a possible involvement of the voltage-activated formation of inositol trisphosphate (IP₃) has been considered (Suzuki et al., 2000; Suzuki, 2000; Hirst and Ward, 2003). These results suggest that flufenamic acid inhibits the depolarization-activated formation of IP₃ production. Alternatively, the increase by flufenamic acid of the latency for the 2nd component is causally related to the reduced activation of Ca²⁺-sensitive Cl⁻-channels.

It is concluded that in antrum muscles of the stomach, flufenamic acid selectively inhibits the activity of the circular muscle layer including ICC-IM, mainly by inhibiting Ca²⁺-sensitive Cl⁻-channels. The inhibitory effects of flufenamic acid on pacemaker potentials are very weak, and as a consequence the inhibition occurs mainly on the amplitude but not the frequency of the slow waves. The nifedipine-sensitive Ca²⁺-channels are not inhibited by flufenamic acid.
Acknowledgments

The present experiments were supported by a Grant-in-Aid for Scientific Research (C) (17590190) from the Japan Society for the Promotion of Science (to H.S.).

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(Received May 17, 2005; Accepted June 30, 2005)