Fenamates Potentiate the α_1-Adrenoceptor-Activated Nonselective Cation Channels in Rabbit Portal Vein Smooth Muscle

Kazunori Yamada, Yoshiki Waniishi, Ryuji Inoue* and Yushi Ito

Department of Pharmacology, Faculty of Medicine, Kyushu University, Fukuoka 812-82, Japan

Received October 16, 1995 Accepted November 7, 1995

ABSTRACT—The agonistic action of fenamates on the α_1-adrenoceptor-activated cationic current (I_{cat}) in rabbit portal vein smooth muscle was investigated with the whole-cell patch clamp technique. At −50 mV, the fenamates (100-500 μM) increased I_{cat} dose-dependently, up to several fold. This increase was not accompanied by changes in the reversal potential and strongly inhibited by 500 μM Cd^{2+} or 10 mM procaine. The enhancing effect of fenamates was also observed on the cationic current activated by intracellularly applied GTPγS. These results suggest that fenamates may be useful as a new class of activator for receptor-operated cation channels in smooth muscle.

Keywords: Cation channel opener, Smooth muscle, Fenamate

Nonselective cation channels (NSCCs) constitute a large heterogeneous family of transmembrane channels that allow nonspecific passage of cations and open in response to a wide variety of stimuli imposed on the cell membrane. Despite their postulated significance in various cellular functions, the pharmacology of these channels remains poorly elucidated (1).

Fenamates such as mefenamate and flufenamate are derivatives of diphenylamine-2-carboxylate (DPC) and have emerged as non-steroidal antiinflammatory drugs. Although early electrophysiological studies reported that these compounds inhibit anionic movements, e.g., through a band 3 anion transporter in erythrocytes and Ca^{2+}-activated Cl^- channels in Xenopus oocytes (for review, see ref. 1), they were later shown to block NSCCs at micromolar concentrations in pancreatic exocrine gland (2). Since then, fenamates have been regarded as blockers for NSCCs in various tissues including smooth muscle (3).

In this paper, we describe a novel action of fenamates on NSCCs, i.e., their activation of the α_1-adrenoceptor-activated cation channels in rabbit portal vein smooth muscle. This seems to have considerable pharmacological importance, because no chemicals are so far known to activate NSCCs except for naturally occurring marine toxins such as maitotoxin and palytoxin.

Male albino rabbits (1.8-2.0 kg, Japanese White; Kajitani, Fukuoka) were anesthetized with intravenous sodium pentobarbitone (40 mg/kg) and then exsanguinated. A segment of the portal vein was excised after opening the abdominal cavity. The details for cell dispersion and the equipment and procedures employed in the patch clamp experiments are described elsewhere (4, 5). For quantifying changes in the amplitude of small noisy cationic currents, the current amplitude was integrated over 10 sec and averaged as the charge transported per second.

Solutions: modified Krebs solution: 140 mM Na^+, 5 mM K^+, 1.0 mM Ca^{2+}, 1.2 mM Mg^{2+}, 149.4 mM Cl^-, 5 mM glucose, 10 mM Hepes/Tris (pH 7.35-7.4); pipette solution: 130 mM Cs^+, 2 mM Mg^{2+}, 20 mM Cl^-, 110 mM aspartate$^-$, 2 mM SO_4^{2-}, 10 mM EGTA and 10 mM Hepes (titrated to 7.2 with Tris base). The liquid junction potential of the internal solution with regard to the modified Krebs solution (−6 mV) was corrected on later analysis.

Chemicals: Mefenamate and flufenamate were purchased from Wako Pure Chemical Industries, Ltd. (Osaka). All drugs, dissolved in DMSO as 200-500 mM stock solutions, were freshly diluted in the bathing solution just before use.

All statistical data represent means±S.E.M.

Figures 1Aa and 2Ba are typical records demonstrating the effects of fenamates on phenylephrine (phe)-induced (α_1-adrenoceptor-activated) inward current (I_{cat}; 4, 6). Addition of either mefenamate or flufenamate into the

* To whom correspondence should be addressed.

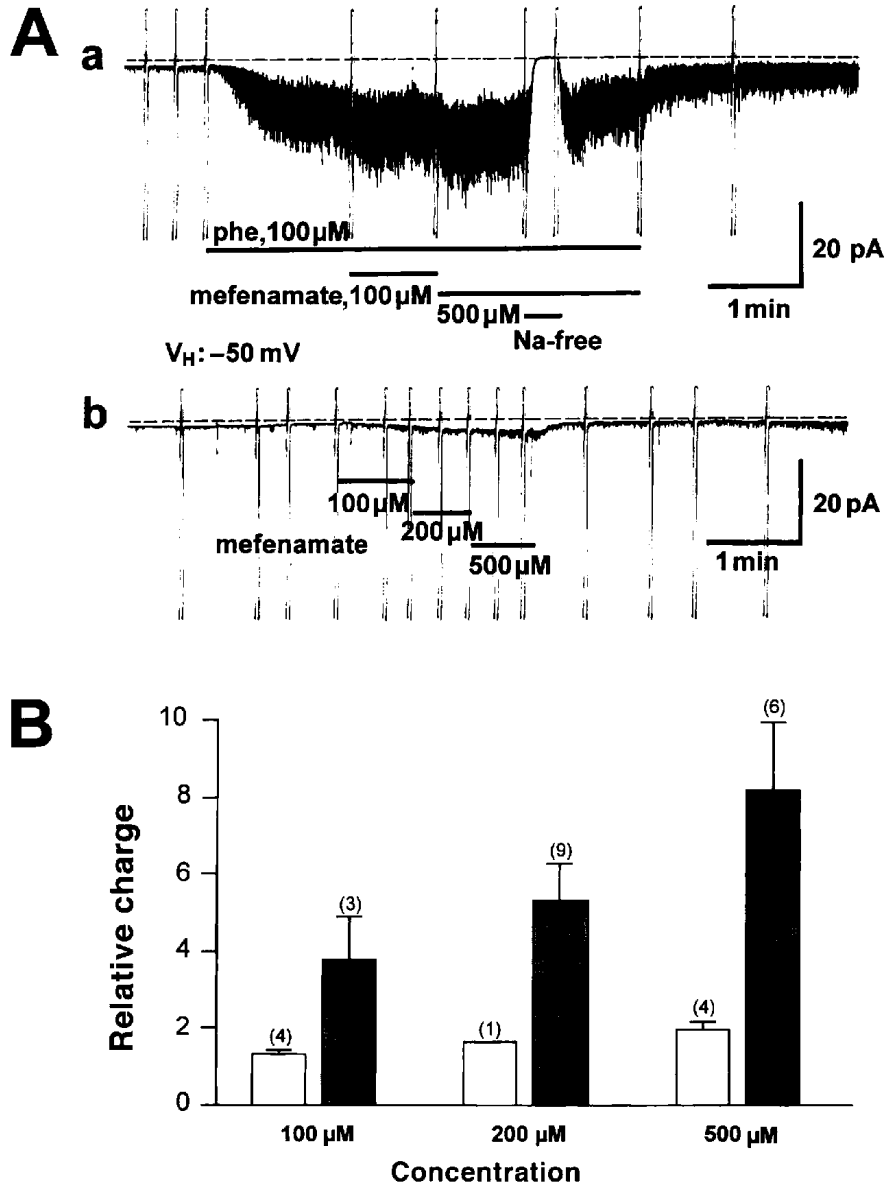

Fig. 1. Fenamates potentiate phenylephrine (phe)-induced cationic currents. A: Effects of mefenamate on the membrane current (at a holding potential of −50 mV) in the presence (a) and absence (b) of 100 μM phe, from the same cell. In Na^+-free solution, all cations except for protons were substituted by equimolar *N*-methyl D-glucamine. A slowly progressive decline in I_{cat} was observed in the continued presence of phe and/or fenamates. Dashed lines indicate the zero current level. B: Summary of fenamate-induced potentiation of I_{cat} (in the presence of 100 μM phe). Open column, mefenamate; filled column, flufenamate. Columns and bars indicate the mean±S.E.M., respectively. The charge carried through I_{cat} at a given concentration of fenamate is normalized to that in its absence. The numbers in parentheses are the number of experiments.

bath produced a dose-dependent increase in the amplitude of inward currents in the presence of phe, which was completely abolished upon substituting external cations with *N*-methyl D-glucamine. The main part of this increase is unlikely to result from direct activation of the cationic current by the fenamates, since they could occasionally induce only small inward currents in the absence of phe (Fig. 1Ab: at −50 mV, 0.3±0.2 pA in 2 out of 6 cells and 0.9±0.4 pA in 2 out of 4 cells with 200 and 500 μM mefenamate, respectively, and 0.9±0.3 pA in 9 out of 16 cells and 2.2±0.4 pA in 7 out of 10 cells with 200 and 500 μM flufenamate, respectively). The percent increase of I_{cat} attained by 500 μM fenamates was 195±24% (n=4) and 818±177% (n=6) for mefenamate and flufenamate, respectively, suggesting that flufenamate is fourfold more potent than mefenamate (Fig. 1B).

The enhancing effect of fenamates on I_{cat} was still observed when the external cation was substituted by a sin-

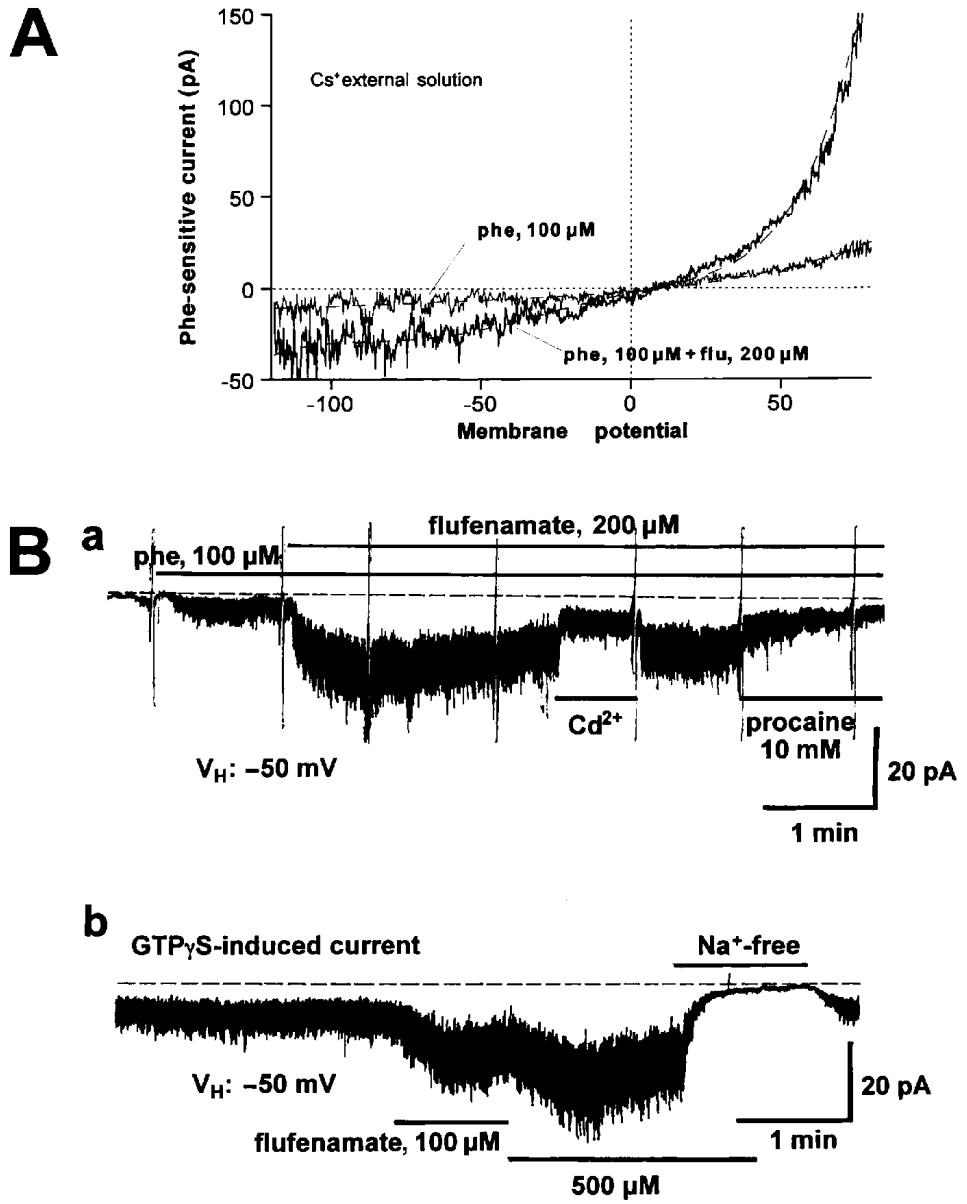

Fig. 2. Properties of cationic currents after potentiation by flufenamate (flu). A: Current-voltage-relationship of I_{cat} under conditions where external cations were substituted by equimolar Cs^+. Voltage ramps of 2 sec (−120 to 80 mV) were applied with and without phenylephrine (phe), and the net current obtained from the latter was subtracted from that obtained from the former. Dashed curves in panel A are drawn according to the best fit of data with 4th- to 5th-order polynomials from which the reversal potentials were measured. Ba: Inhibition of I_{cat} by 500 μM Cd^{2+} and 10 mM procaine. Bb: Flufenamate caused a dose-dependent increase in the cationic current induced by 50 μM GTPγS. Dashed lines in panel B indicate the zero-current level.

gle cation, Cs^+, and the reversal potential of I_{cat} was little affected after potentiation by fenamates (Fig. 2A: the shift of reversal potential was -0.3 ± 0.7 mV in Cs^+ external solution (n=4) and 0.1 ± 0.4 mV in modified Krebs solution (n=5)). These results suggest that the large increment induced by fenamates may not result from the altered ionic permeability of the phe-activated cation channels.

The degree of increase in I_{cat} by fenamates appeared to depend on the membrane potential (Fig. 2A): the percentage increase induced by 200 μM flufenamate at −50, 50 and 75 mV were 317%, 375% and 659%, respectively. This suggests that membrane depolarization might potentiate the fenamate-induced increase in the cationic current. Addition of potent blockers for receptor-operated cationic channels, 500 μM Cd^{2+} or 10 mM procaine (3,

4), strongly inhibited the fenamate-induced increase in I_{cat} (Fig. 2Ba).

The major site of action of fenamates may be located downstream from the α_1-adrenoceptor, since the degree of increase in I_{cat} did not significantly alter with different concentrations of phe or even when a muscarinic agonist carbachol (4) was used to activate I_{cat} (data not shown). Furthermore, fenamates caused a similar increase (2.2 ± 0.2 (n=4)- and 3.8 ± 0.3 (n=3)-fold with 200 μM mefenamate and flufenamate, respectively) in the small noisy cationic currents that were induced by adding 50 μM GTPγS into the patch pipette (15.0 ± 2.1 pA at -50 mV, n=5) bypassing the process of ligand-receptor binding (Fig. 2Bb), although whether the properties of this cationic current are identical to those of I_{cat} requires further investigation.

The present results have revealed that fenamates, which have been regarded as general blockers for various types of NSCCs (1), could act agonistically for a certain type of NSCC, i.e., the α_1-adrenoceptor-activated cation channels in portal vein smooth muscle.

Literally, there are so far only two marine toxins known to activate the nonselective cation channels without mediation of cell surface receptors or intracellular messengers. Maitotoxin, a water-soluble polyether, has been reported to stimulate Ca^{2+} entry into the cell by opening 40 pS cation-selective channels in renal epithelial cells (7), and another non-peptide toxin, palytoxin, is found to depolarize the membrane due to activation of 10 pS monovalent cation permeable channels in cardiac myocytes (8). However, the mechanisms underlying the agonistic effects of fenamates seem basically different from those of these toxins. First, fenamates seem to need prior activation of cation channels through α_1-adrenoceptors to exert their agonistic actions, although marginally small inward currents were sometimes detected in the sole presence of fenamates. Secondly, their duality to behave both antagonistically and agonistically depending on the type of NSCCs is certainly a notable difference. These actions of fenamates, together with the fact that the cationic currents activated by internal perfusion of GTPγS or by the agonists related to distinct receptors and G-proteins (i.e., phe and carbachol) also show similar sensitivities to fenamates, imply close association of fenamate's action with the cation channel per se, and thus may be reminiscent of those of dihydropyridines such as nifedipine and BAY K 8644 on the high-threshold voltage-dependent Ca^{2+} channels, where partial modification of side chains or differences in the spatial configurations of these compounds significantly affect their antagonistic or agonistic properties (e.g., 9).

It is conceivable that whether fenamates act antagonistically or agonistically might depend on subtle differences in the cation channel architecture, since the muscarinic receptor cation channel in guinea pig ileal smooth muscle, which resembles the cation channel of α_1-adrenoceptor in many respects (4), is potently blocked by fenamates (3). Hence, fenamates might serve as a useful starting point to design agonists and antagonists for the receptor-operated nonselective cation channels in smooth muscle. Further efforts are needed to investigate more details of the actions of fenamates, since these compounds have also been reported to activate certain types of K^+ channels in recent studies (e.g., 10).

Acknowledgments

We heartily thank Dr. A.F. Brading for improving our manuscript.

REFERENCES

1 Hescheler J and Schultz G: Nonselective cation channels: Physiological and pharmacological modulations of channel activity. *In* Nonselective Cation Channels, Edited by Siemen D and Hescheler J, pp 27–43, Birkhäuser, Basel (1993)
2 Gögelein H, Dahlem D, Engert HC and Lang HJ: Flufenamic and mefenamic acid inhibit single nonselective cation channels in the rat exocrine pancreas. FEBS Lett **268**, 79–82 (1990)
3 Chen S, Inoue R and Ito Y: Pharmacological characterization of muscarinic receptor-activated cation channels in guinea pig ileum. Br J Pharmacol **109**, 793–801 (1993)
4 Inoue R and Kuriyama H: Dual regulation of cation-selective channels by muscarinic and α_1-adrenergic receptors in the rabbit portal vein. J Physiol (Lond) **465**, 427–448 (1993)
5 Inoue R, Waniishi Y, Yamada K and Ito Y: Extracellular H^+ modulates acetylcholine-activated nonselective cation channels in guinea-pig ileum. Am J Physiol **268**, C162–C170 (1995)
6 Wang Q and Large WA: Noradrenaline-evoked cation conductance recorded with the nystatin whole-cell method in rabbit portal vein cells. J Physiol (Lond) **435**, 21–39 (1991)
7 Dietl P and Volkl H: Maitotoxin activates a nonselective cation channel and stimulates Ca^{2+} entry in MDCK renal epithelial cells. Mol Pharmacol **45**, 300–305 (1994)
8 Ikeda M, Mitani K and Ito K: Palytoxin induces a nonselective cation channel in single ventricular cells of rat. Naunyn Schmiedebergs Arch Pharmacol **337**, 591–593 (1988)
9 Hille B: Ionic Channels of Excitable Membranes, 2nd Edition, Sinauer Associates Inc, Sunderland (1992)
10 Farrugia G, Rae JL and Szurszewski JH: Characterization of an outward potassium current in canine jejunal circular smooth muscle and its activation by fenamates. J Physiol (Lond) **468**, 297–310 (1993)