

• BASIC RESEARCH •

Effects of unsaturated fatty acids on calcium-activated potassium current in gastric myocytes of guinea pigs

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Abstract

AIM: To investigate the effects of exogenous unsaturated fatty acids on calcium-activated potassium current [$I_{K(Ca)}$] in gastric antral circular myocytes of guinea pigs.

METHODS: Gastric myocytes were isolated by collagenase from the antral circular layer of guinea pig stomach. The whole-cell patch clamp technique was used to record $I_{K(Ca)}$ in the isolated single smooth muscle cells with or without different concentrations of arachidonic acid (AA), linoleic acid (LA), and oleic acid (OA).

RESULTS: AA at concentrations of 2.5 and 10 $\mu\text{mol/L}$ markedly increased $I_{K(Ca)}$ in a dose-dependent manner. LA at concentrations of 5, 10 and 20 $\mu\text{mol/L}$ also enhanced $I_{K(Ca)}$ in a dose-dependent manner. The increasing potency of AA, LA, and oleic acid (OA) on $I_{K(Ca)}$ at the same concentration (10 $\mu\text{mol/L}$) was in the order of $\text{AA} > \text{LA} > \text{OA}$. AA (10 $\mu\text{mol/L}$)-induced increase of $I_{K(Ca)}$ was not blocked by H-7 (10 $\mu\text{mol/L}$), an inhibitor of protein kinase C (PKC), or indomethacin (10 $\mu\text{mol/L}$), an inhibitor of the cyclooxygenase pathway, and 17-octadecynoic acid (10 $\mu\text{mol/L}$), an inhibitor of the cytochrome P450 pathway, but weakened by nordihydroguaiaretic acid (10 $\mu\text{mol/L}$), an inhibitor of the lipoxygenase pathway.

CONCLUSION: Unsaturated fatty acids markedly increase $I_{K(Ca)}$, and the enhancing potencies are related to the number of double bonds in the fatty acid chain. The lipoxygenase pathway of unsaturated fatty acid metabolism is involved in the unsaturated fatty acid-induced increase of $I_{K(Ca)}$ in gastric antral circular myocytes of guinea pigs.

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Key words: Gastric myocytes; Calcium-activated potassium channel; Unsaturated fatty acids

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INTRODUCTION

Unsaturated fatty acids are the major components of membrane lipids and they are mainly released by stimulation of phospholipase A_2 . Arachidonic acid (AA) and other unsaturated fatty acids modulate the activities of various ion channels^[1-3] through direct or indirect pathways. The direct effects are mediated by the interaction between fatty acids and ion channel proteins or through the interference with plasma membranes. The indirect actions on ion channels result from cyclo-oxygenase, lipoxygenase, and epoxigenase metabolites or cellular signal transduction pathways^[4]. For example, AA directly affects the activities of cloned human potassium channels mainly existing in heart and brain^[5] and Ca^{2+} -activated K^+ channels in rabbit coronary smooth muscle cells^[6]. In addition, AA has been shown to modulate ion transient receptor potential channels as a second messenger^[7] and to enhance voltage-dependent calcium channels in vascular smooth muscle cells through cytochrome P450 metabolites^[8].

The Ca^{2+} -activated potassium channel [$I_{K(Ca)}$] has been considered to play an important role in excitability and functional regulation in excitable cells^[9]. Agonists of $I_{K(Ca)}$, such as carbon monoxide and bradykinin, which change the activity of the Ca^{2+} -activated potassium channels, can affect the membrane potential and contractility in smooth muscle cells^[10,11]. We have shown that NO relaxes gastric antral smooth muscle of the guinea pig through increase of $I_{K(Ca)}$ ^[12]. It has been reported that AA affects $I_{K(Ca)}$ in many cells. It inhibits $I_{K(Ca)}$ in T84 cells^[13], activates $I_{K(Ca)}$ in vascular smooth muscles^[14] and GH(3) cells^[15]. In our previous study, we have reported that AA and other unsaturated fatty acids directly inhibit calcium current (I_{Ca})^[16], chloride current (I_{Cl})^[17] and muscarinic current (I_{CCh})^[18] in gastric myocytes of guinea pigs. But the effects of AA and other unsaturated fatty acids on $I_{K(Ca)}$ in gastric myocytes have not yet been reported. In the present study, we investigated the effect of AA and other unsaturated fatty acids on $I_{K(Ca)}$ in gastric antral circular myocytes of guinea pigs.

MATERIALS AND METHODS

Preparation of cells

Gastric myocytes were isolated enzymatically from the antral circular layer of guinea pig stomachs as described previously^[15]. Briefly, EWG/B guinea pigs (obtained from the Experimental Animal Department of Jilin University Clinical College, Certificate No 10-6004) of either sex weighing 300-350 g were euthanized by a lethal dose of IV sodium pentobarbital (50 mg/kg). The antral part of the stomach was dissected from the longitudinal layer using fine scissors and then cut into small segments (2-3 mm). The tissue chunks were then incubated at 36 °C for 25-30 min in a digestion medium consisting of 4 mL Ca^{2+} -free physiology solution containing 8 mg bovine serum albumin, 4.5 mg trypsin inhibitor, 4 mg collagenase type II, and 4 mg dithioerythritol. Single myocytes were kept at 4 °C until use.

Electrophysiological recordings

The isolated cells were transferred to a small chamber (0.1 mL) on the stage of an inverted microscope (IX-70 Olympus, Japan)

for 10–15 min to settle down. The cells were superfused continuously with isosmotic solution. An 8-channel perfusion system (L/M-sps-8, List Electronics, Germany) was used to change the solution. Experiments were performed at 20–25 °C and the whole-cell configuration of the patch-clamp technique was applied. Patch-clamp pipettes were manufactured from borosilicate glass capillaries (GC 150T-7.5, Clark Electromedical Instruments, UK) by a two-stage puller (PP-83, Narishige, Japan). The resistance of the patch pipettes was 3–5 M Ω when being filled with pipette solution. Liquid junction potentials were compensated prior to seal formation. The whole-cell holding currents were recorded with an Axopatch 1-D patch-clamp amplifier (Axon Instrument, USA) and an EPC-10 amplifier (HEKA Instrument, Germany).

Drugs and solutions

All drugs were purchased from Sigma Chemical Co, USA. Tyrode's solution contained NaCl 147, KCl 4, CaCl₂·2H₂O 2, MgCl₂·6H₂O 1.05, NaH₂PO₄·2H₂O 0.42, Na₂HPO₄·2H₂O 1.81 and glucose 5.5 mmol/L, pH was adjusted to 7.35 with NaOH. PSS contained NaCl 134.8, KCl 4.5, MgCl₂·6H₂O 1.0, CaCl₂·2H₂O 2.0, glucose 5.0 and HEPES 10.0 mmol/L, and pH was adjusted to 7.4 by using Tris. In Ca²⁺-free PSS, 2.0 mmol/L CaCl₂·2H₂O was omitted from PSS. The pH of modified Kraft-Bruhe solution containing 0.5 mmol/L egtazic acid, 10 mmol/L HEPES, MgCl₂·6H₂O 3 mmol/L, 50 mmol/L KCl, 10 mmol/L glucose, 50 mmol/L L-glutamata, 20 mmol/L taurine and 20 mmol/L KH₂PO₄, was adjusted to 7.40 with KOH 1 mmol/L. The pipette solution contained 110 mmol/L potassium-aspartic acid, 5 mmol/L Mg-ATP, 5 mmol/L HEPES, 1.0 mmol/L MgCl₂·6H₂O, 20 mmol/L KCl, 0.1 mmol/L egtazic acid, 2.5 mmol/L di-*tris*-creatine phosphate and 2.5 mmol/L disodium-creatine phosphate, pH was adjusted to 7.30 with Tris. AA, LA and OA were separately prepared at 1 mmol/L. All unsaturated fatty acids were added in external perfusing solution. Indomethacin, 17-octadecynoic acid, nordihydroguaiaretic acid and H-7 were prepared at 1 mmol/L.

Statistical analysis

This experiment was consubstantially compared. The current before perfusion with fatty acids served as controls. All values were expressed as mean \pm SD. Statistical significance was evaluated by *t*-test.

RESULTS

Effects of unsaturated fatty acids on $I_{K(Ca)}$

Under the whole-cell configuration, the membrane potential was clamped at -60 mV, and $I_{K(Ca)}$ was elicited by step voltage command pulse from -40 mV to 100 mV for 440 ms with a 20 mV increment at 10 s intervals. AA, an unsaturated fatty acid (with 4 double bonds) significantly increased $I_{K(Ca)}$ in a dose-dependent manner. AA increased $I_{K(Ca)}$ by (15.9 \pm 3.6)%, (31.9 \pm 7.0)% and (46.3 \pm 10.4)% at the concentrations of 2, 5 and 10 μ mol/L at +60 mV, respectively (n = 8, Figure 1 C). Under the whole-cell patch-clamp mode the membrane potential was clamped at -20 mV, the spontaneous transient outward currents (STOCs) due to activation of calcium-activated potassium^[19] were then recorded. AA markedly increased STOCs at 10 μ mol/L (Figure 1 D). Another unsaturated fatty acid LA (with 2 double bonds) also increased $I_{K(Ca)}$ by (27.8 \pm 4.8)%, (37.9 \pm 13.9)% and (70.8 \pm 19.9)% at the concentrations of 5, 10 and 20 μ mol/L at +60 mV, respectively (n = 8, Figure 1F–G).

Comparison of the effects among different unsaturated fatty acids on $I_{K(Ca)}$

To determine the enhancing potency of unsaturated fatty acids,

the effects of different unsaturated fatty acids on $I_{K(Ca)}$ were observed. Under the whole-cell configuration, AA, LA, and OA (with one double bond) at the same concentration (10 μ mol/L) increased $I_{K(Ca)}$ by (46.3 \pm 10.4)%, (37.9 \pm 13.9)% and (13.5 \pm 5.1)% at +60 mV, respectively (n = 8, Figure 2). Among them, the increasing potency was in the order of AA (C20: 4, *cis*-5, 8, 11, 14) > LA (C18: 2, *cis*-9, 12) > OA (C18: 1, *cis*-9). The increasing potency of unsaturated fatty acids was in accordance with the number of double bonds in the fatty acid chain.

Effects of PKC inhibitor and oxygenase inhibitor on AA-induced increase of $I_{K(Ca)}$

To determine whether unsaturated fatty acids induced increase of $I_{K(Ca)}$ directly or indirectly, the effect of AA on $I_{K(Ca)}$ was observed after pretreatment with indomethacin (indocin, cyclo-oxygenase inhibitor), nordihydroguaiaretic acid (NDGA, lipoxygenase inhibitor), 17-octadecynoic acid (17-ODA, cytochrome P450 inhibitor) and H-7 (protein kinase C inhibitor), which were added in external perfusing solution for about 10–15 min. H-7 (10 μ mol/L), indocin (10 μ mol/L) and 17-ODA (10 μ mol/L) could not block AA-induced increase of $I_{K(Ca)}$, and AA still increased $I_{K(Ca)}$ by (41.8 \pm 3.7)%, (42.9 \pm 10.8)% and (40.8 \pm 6.8)% at +60 mV, respectively (Figure 3). There was no significant difference between the two groups before and after pretreatment with H-7 and oxygenase inhibitors (P < 0.05, n = 8). But after pretreatment with NDGA 10 μ mol/L, AA-induced increase of $I_{K(Ca)}$ was diminished from 46.3 \pm 10.4% of control to (11.3 \pm 4.3)% (Figure 3). There was a significant difference between the two groups before and after pretreatment with NDGA (P > 0.05, n = 8).

DISCUSSION

In this study, it was found that unsaturated fatty acids increased $I_{K(Ca)}$ in a dose-dependent manner and AA increased STOCs also. AA-induced increase of $I_{K(Ca)}$ was not blocked by H-7, indocin and 17-ODA, but was markedly weakened by NDGA. Many experiments have shown that AA and other unsaturated fatty acids enhance $I_{K(Ca)}$. It has been described that AA could directly increase $I_{K(Ca)}$ in human mesangial cells^[20] through lipoxygenase metabolites in rat pituitary tumor cells^[21] and cytochrome p-450 epoxygenase products in smooth muscle cells of rat cerebral arteries^[22]. The results described here show that unsaturated fatty acids increase $I_{K(Ca)}$ and the more double bonds they have, the more potent their enhancing effect on $I_{K(Ca)}$ in gastric antral smooth muscle cells of guinea pigs is. Our previous studies have shown that more double bonds lead to more inhibitory potency on I_{Ca} ^[16], and I_{CCh} ^[18] in gastric antral smooth muscle cells of guinea pigs; however, saturated fatty acids have no effect on I_{Cl} ^[17]. Horimoto *et al.*^[23] also reported only fatty acids having more than two double bonds activated the K⁺ channels in freshly dissociated neurons of 10- to 20-day-old rat visual cortex. These data show that double bonds must be satisfied for a given fatty acid to affect ion channels. The double bonds of unsaturated fatty acids might be easily oxidized to form reactive oxygen species or make unsaturated fatty acids to form barrette-like structures, which may optimize the possibility of binding to ion channels to modulate $I_{K(Ca)}$ ^[15].

The indirect effects of AA on ion channels require the metabolite transformation of AA^[20,21] and activation of PKC^[24]. In this study, the lipoxygenase metabolism pathway was involved in AA-induced increase of $I_{K(Ca)}$, since NDGA markedly diminished AA-induced increase of $I_{K(Ca)}$, but H-7, indocin and 17-ODA had no effect. Many studies have demonstrated that AA exerts physiological function via lipoxygenase metabolism pathway by modulating ion channels. It has been reported that

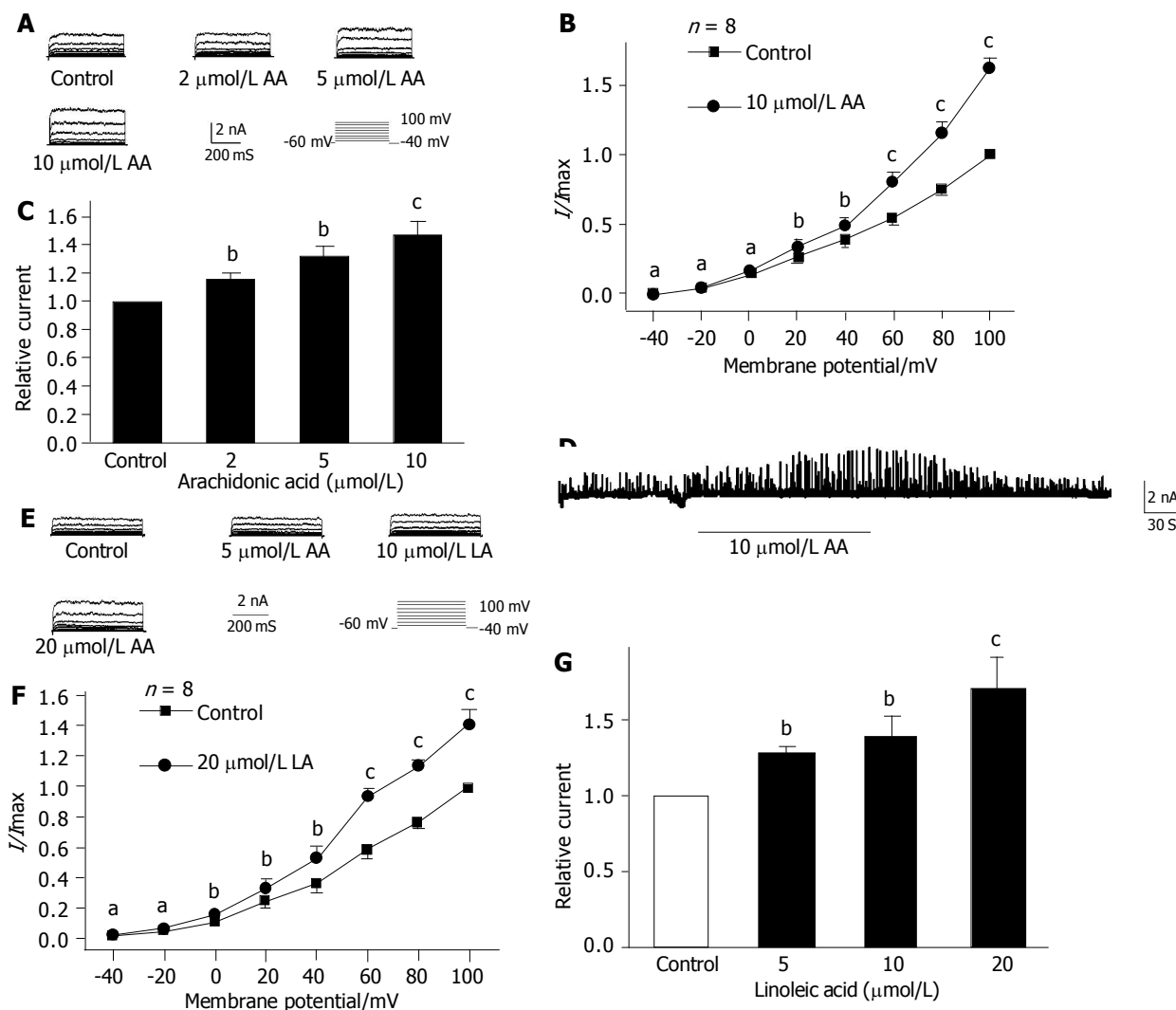


Figure 1 Effects of AA and LA on $I_{K(Ca)}$. A: Raw traces of AA on $I_{K(Ca)}$ at different concentrations; B: I/V relationship of AA on $I_{K(Ca)}$. Peak values were normalized to the values obtained at 100mV under control condition ($n = 8$, $^aP > 0.05$, $^cP < 0.05$, $^bP < 0.01$ vs control); C: Dose-dependent increase of AA on $I_{K(Ca)}$ ($n = 8$, $^cP < 0.05$, $^bP < 0.01$ vs control); D: Increase of AA on STOCs; E: Raw traces of LA on $I_{K(Ca)}$ at different concentrations; F: I/V relationship of LA on $I_{K(Ca)}$ ($n = 8$, $^aP > 0.05$, $^cP < 0.05$, $^bP < 0.01$ vs control); G: Dose-dependent increase of LA on $I_{K(Ca)}$ ($n = 8$, $^aP < 0.05$, $^bP < 0.01$ vs control).

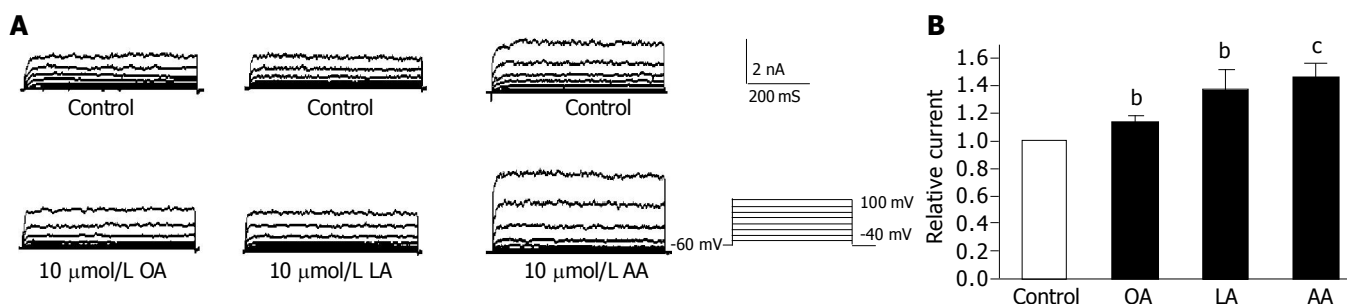


Figure 2 Comparison of different unsaturated fatty acids on $I_{K(Ca)}$. A: Raw traces of 10 μmol/L OA, LA and AA on $I_{K(Ca)}$; B: Increased effect of different unsaturated fatty acids on $I_{K(Ca)}$ ($n = 8$, $^aP < 0.05$, $^bP < 0.01$ vs control).

the lipoxygenase pathway mediates AA-induced vasodilation through a K^+ channel-dependent mechanism in rat small mesenteric arteries and rat basilar arteries. The effect of AA by lipoxygenase metabolites on $I_{K(Ca)}$ might play an important role in regulating secretory function of adrenal chromaffin cells in bovine. However, we can not exclude the direct effect of AA on $I_{K(Ca)}$, since NDGA could not abolish entirely AA-induced

increase of $I_{K(Ca)}$. Unsaturated fatty acids may directly or/and indirectly modulate $I_{K(Ca)}$.

In summary, $I_{K(Ca)}$ is increased by unsaturated fatty acids in a dose-dependent manner. There is a correlation between the degree of *cis* unsaturation and the increasing potency on $I_{K(Ca)}$. Lipoxygenase metabolism pathway is involved in unsaturated fatty acid-induced increase of $I_{K(Ca)}$.

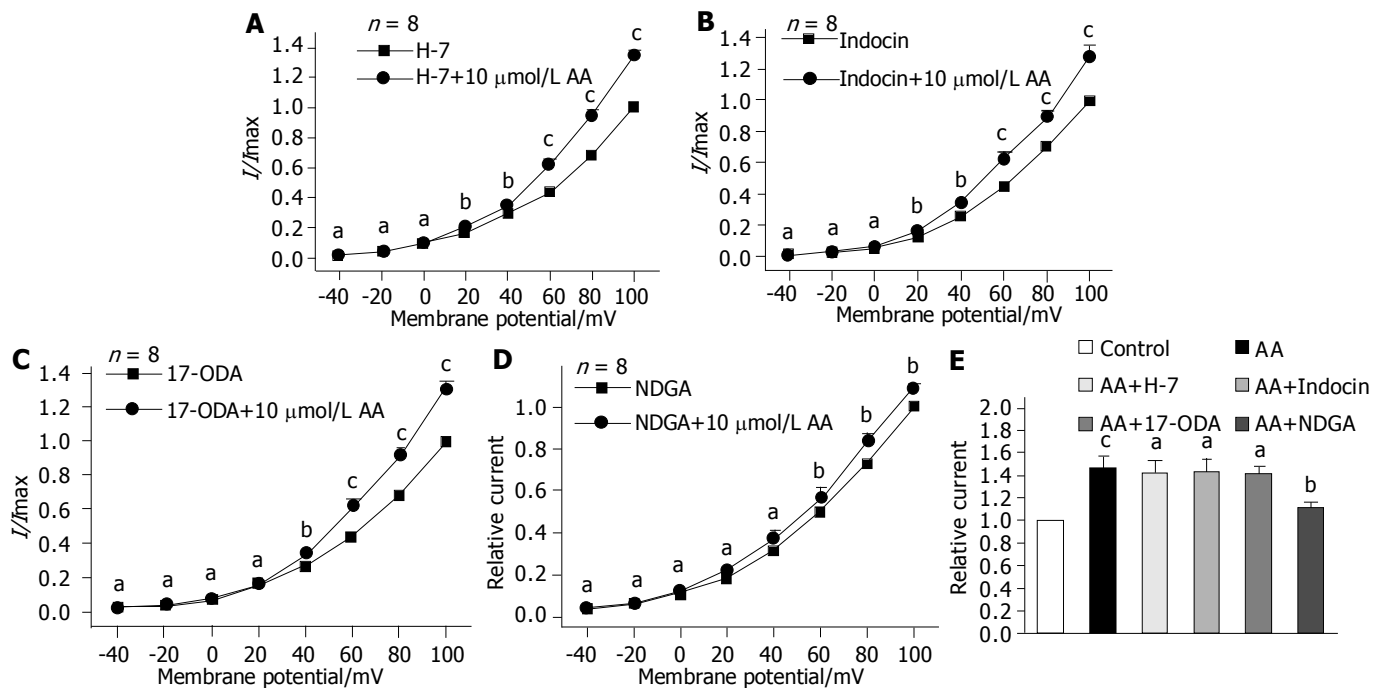


Figure 3 Effects of PKC inhibitor and oxygenase inhibitor on AA-induced increase of $I_{K(Ca)}$. A, B, C and D: Effects of AA on $I_{K(Ca)}$ after pretreatment with H-7, indomethacin, 17-octadecynoic acid and nordihydroguaiaretic acid, respectively ($n = 8$, $^aP > 0.05$, $^cP < 0.05$, $^bP < 0.01$ vs control); E: Comparison of AA on $I_{K(Ca)}$ before and after pretreatment with H-7, indomethacin, 17-octadecynoic acid and nordihydroguaiaretic acid, respectively ($n = 8$, $^bP < 0.01$ vs control, $^aP > 0.05$, $^cP < 0.05$ vs AA).

REFERENCES

- Kang JX, Leaf A. Evidence that free polyunsaturated fatty acids modify Na^+ channels by directly binding to the channel proteins. *Proc Natl Acad Sci USA* 1996; **93**: 3542-3546
- Petit-Jacques J, Hartzell HC. Effect of arachidonic acid on the L-type calcium current in frog cardiac myocytes. *J Physiol* 1996; **493**(Pt 1): 67-81
- Kim D, Pleumsamran A. Cytoplasmic unsaturated free fatty acids inhibit ATP-dependent gating of the G protein-gated K^+ channel. *J Gen Physiol* 2000; **115**: 287-304
- Ordway RW, Singer JJ, Walsh JV. Direct regulation of ion channels by fatty acids. *Trends Neurosci* 1991; **14**: 96-100
- Liu Y, Liu D, Heath L, Meyers DM, Krafte DS, Wagoner PK, Silvia CP, Yu W, Curran ME. Direct activation of an inwardly rectifying potassium channel by arachidonic acid. *Mol Pharmacol* 2001; **59**: 1061-1068
- Ahn DS, Kim YB, Lee YH, Kang BS, Kang DH. Fatty acids directly increase the activity of $Ca(2+)$ -activated K^+ channels in rabbit coronary smooth muscle cells. *Yonsei Med J* 1994; **35**: 10-24
- Hardie RC. Regulation of TRP channels via lipid second messengers. *Annu Rev Physiol* 2003; **65**: 735-759
- Fang X, Weintraub NL, Stoll LL, Spector AA. Epoxyeicosatrienoic acids increase intracellular calcium concentration in vascular smooth muscle cells. *Hypertension* 1999; **34**: 1242-1246
- Lingle CJ. Setting the stage for molecular dissection of the regulatory components of BK channels. *J Gen Physiol* 2002; **120**: 261-265
- Wang R, Wang Z, Wu L. Carbon monoxide-induced vasorelaxation and the underlying mechanisms. *Br J Pharmacol* 1997; **121**: 927-934
- Mazzucco TL, Andre E, Calixto JB. Contribution of nitric oxide, prostanooids and $Ca(2+)$ -activated K^+ channels to the relaxant response of bradykinin in the guinea pig bronchus *in vitro*. *Naunyn Schmiedeberg's Arch Pharmacol* 2000; **361**: 383-390
- Li Y, Xu WX, Li ZL. Effects of nitroprusside, 3-morpholino-sydnominine, and spermine on calcium-sensitive potassium currents in gastric antral circular myocytes of guinea pig. *Acta Pharmacol Sin* 2000; **21**: 571-576
- Devor DC, Frizzell RA. Modulation of K^+ channels by arachidonic acid in T84 cells. I. Inhibition of the $Ca(2+)$ -dependent K^+ channel. *Am J Physiol* 1998; **274**: C138-C148
- Quignard JF, Chataigneau T, Corriu C, Edwards G, Weston A, Feletou M, Vanhoutte PM. Endothelium-dependent hyperpolarization to acetylcholine in carotid artery of guinea pig: role of lipoxygenase. *J Cardiovasc Pharmacol* 2002; **40**: 467-477
- Denson DD, Wang X, Worrell RT, Eaton DC. Effects of fatty acids on BK channels in GH (3) cells. *Am J Physiol Cell Physiol* 2000; **279**: C1211-1219
- Xu WX, Kim SJ, So I, Suh SH, Kim KW. Effects of Arachidonic acid on the calcium channel current (I_{Ba}) and on the osmotic stretch-induced increase of I_{Ba} in guinea pig gastric myocytes. *Korea J physiol pharmacol* 1997; **1**: 435-443
- Xu WX, Kim SJ, So I, Kang TM, Rhee JC, Kim KW. Volume-sensitive chloride current activated by hyposmotic swelling in antral gastric myocytes of the guinea-pig. *Pflügers Arch* 1997; **435**: 9-19
- Cui YF, Jin H, Guo HS, Li L, Yu YC, Xu WX. Effect of unsaturated fatty acid on muscarinic current in guinea pig gastric antral circular myocytes. *Acta Pharmacol Sin* 2003; **24**: 283-288
- Zhuge R, Fogarty KE, Tuft RA, Walsh JV. Spontaneous transient outward currents arise from microdomains where BK channels are exposed to a mean $Ca(2+)$ concentration on the order of 10 microM during a $Ca(2+)$ spark. *J Gen Physiol* 2002; **120**: 15-27
- Stockand JD, Silverman M, Hall D, Derr T, Kuback B, Sansom SC. Arachidonic acid potentiates the feedback response of mesangial BKCa channels to angiotensin II. *Am J Physiol* 1998; **274**: F658-F664
- Duerson K, White RE, Jiang F, Schonbrunn A, Armstrong DL. Somatostatin stimulates BKCa channels in rat pituitary tumor cells through lipoxygenase metabolites of arachidonic acid. *Neuropharmacology* 1996; **35**: 949-961
- Lauterbach B, Barbosa-Sicard E, Wang MH, Honeck H, Kargel E, Theuer J, Schwartzman ML, Haller H, Luft FC, Gollasch M, Schunck WH. Cytochrome P450-dependent eicosapentaenoic acid metabolites are novel BK channel activators. *Hypertension* 2002; **39**: 609-613
- Horimoto N, Nabekura J, Ogawa T. Arachidonic acid activation of potassium channels in rat visual cortex neurons. *Neuroscience* 1997; **77**: 661-671
- Smirnov SV, Aaronson PI. Modulatory effects of arachidonic acid on the delayed rectifier K^+ current in rat pulmonary arterial myocytes. Structural aspects and involvement of protein kinase C. *Circ Res* 1996; **79**: 20-31