Effect of unsaturated fatty acid on muscarinic current in guinea pig gastric antral circular myocytes[1]

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ABSTRACT

AIM: To investigate the effect of ectogenesis unsaturated fatty acid on carbachol-induced muscarinic current (I_{CCh}) and its mechanism. **METHODS:** Using the whole-cell patch-clamp technique, I_{CCh} was recorded in single smooth muscle cell isolated from the antral circular smooth mucles of guinea-pig stomach. **RESULTS:** Arachidonic acid (AA) was added in external perfusing solution and AA inhibited I_{CCh} to 46 %±8 %, 23 %±5 %, and 3.8 %±0.9 % at 1, 3, and 5 μmol/L. Another unsaturated fatty acid, linoleic acid (LA) also inhibited I_{CCh} in a dose-dependant manner. LA inhibited I_{CCh} to 69 %±10 %, 35 %±5 %, and 7.4 %±1.2 % at 1, 5, and 10 μmol/L, respectively. The same concentration (5 μmol/L) of AA, LA, and oleic acid (OA) suppressed I_{CCh} to 3.8 %±0.9 %, 35 %±5 %, and 67 %±9 %, respectively. The inhibitory potency sequence of these unsaturated fatty acids was AA>LA>OA. After 10-15 min of pretreatment with H-7 (a protein phosphorylation C inhibitor) 100 μmol/L or indomethacin (a cyclooxygenase inhibitor) 10 μmol/L, I_{CCh} was inhibited by 5 μmol/L of AA to 5.5 %±0.7 % and 3.0 %±1.0 %, respectively. **CONCLUSION:** The unsaturated fatty acids directly inhibited I_{CCh}, and the inhibitory potency was related to the number of double bonds in fatty acid chain.

INTRODUCTION

Unsaturated fatty acids are a major component of cell membrane phospholipid[1], arachhidonic acid (AA) and other unsaturated fatty acids can be liberated from cell membranes either through a direct action of phospholipase A_2 or through the combined action of phospholipase C and diacylglycerol lipase. AA is an important second messanger in a wide variety of cell types[2], and a number of AA metabolites from cyclooxygenase and lipoxygenase pathways mediate variety of cell signaling events[3,4].

The effects of fatty acid on ion channel are complicated: some ion channels are affected by both saturated and unsaturated fatty acid while others are affected only by unsaturated fatty acids; some ion channels are affected by both of the *cis*- and *trans*-forms of unsaturated fatty acids whereas others require the *cis*-configration to produce an effect[5-7]. The diversity of effects at least in part arises because of several alternative ways that fatty acid could alter channel activity. First, fatty acid could interact directly with a channel protein at a fatty acid binding site to alter channel activity; Second, fatty acid could interact with a membrane lipid to change membrane structure and fluidity, thereby altering channel activity; Third, the double bonds of un-

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saturated fatty acids are easily oxidized to form reactive oxygen species that can affect channel function; Finally, many fatty acids can be metabolized to form reactive products, but which them can alter channel function were not clear[7]. Recently, the effects of AA on variety ion channels have been reported. AA inhibited L-type and N-type Ca^{2+} channel in rat sympathetic[8]. But AA reversiberly enhanced N-type calcium current at an extracellur site[9]. AA directly and non-selectly inhibited both K^+ and Ca^{2+} currents in isolated type I cells of the rat carotid body[10]. Our previous study also observed that AA directly inhibited calcium current[11] and Cl- current[12] in guinea pig gastric myocyte. But the effects of AA and other unsaturated fatty acids on muscarinic current (ICCh) in gastric myocytes have not yet been reported. So in this study we observed the effect of AA and other unsaturated fatty acids on ICCh and its possible mechanism.

MATERIALS AND METHODS

Cell dissociation Gastric myocytes were isolated enzymatically from the antral circular layer of guinea pig stomach as described previously[13]. Briefly, EWG/B guinea pigs (obtained from the Experimental Animal Department of Norman Bethune University, Certificate No 10-6004) of either sex weighing 300-350 g were euthanized by lethal dose of iv pentobarbital sodium (50 mg/kg). The antral part of the stomach was dissected from the logitudinal layer using fine scissors and then cut into small segements (2-3 mm). The tissue chunks were then incubated at 36 ° C for 25-30 min in a digestion medium consisting of Ca^{2+}-free physiology solution containing bovine serum albumin 8 mg, trypsin inhibitor 4.5 mg, collagenase type 2 4 mg , and dithioerythreitol 4 mg. Single myocytes were kept at 4 ° C until use.

Electrophysiological recordings Whole-cell patch-clamp technique was adopted to hold the membrane potential at -20 mV, using an Axo patch 1-D patch-clamp amplifier (Axon Instrument, USA). An aliquot of single smooth muscle cells in suspension was added to recording chamber (0.3 mL) mounted on an inverted microscope (IX-70; Olympus, Optical, Japan). Solutions were perfused at a speed of 2 mL/min through the chamber by gravity at a rate of approx from the 8-channel perfusion system (L/M-sps-8; List Electronics, Germany). Command pluses were applied with the IBM-compitable 486-grade computer and whole cell currents were recorded with a pen recorder. (RM-6220; Japan). In this experiment, after external perfusing with carbachol (50 μmol/L), muscarinic current was activated at about 50 s and achieved to steady state in about 1 min. When the muscarinic current was achieved to steady state unsaturated fatty acids were added in external perfusing solution. The effects of unsaturated fatty acids on muscarinic current appeared at about 50 s and achieved to steay state in 1 min. When the effects of fatty acids were achieved to steady state the external perfusing solution (containing fatty acids) was exchanged to normal perfusing solution.

Drugs and solutions All drugs were purchased from Sigma Chemical Co, USA. Tyrode's solution contained NaCl 147, KCl 4, $CaCl_2 \cdot 2H_2O_2$, $MgCl_2 \cdot 6H_2O$ 1.05, $NaH_2PO_4 \cdot 2H_2O$ 0.42, $Na_2HPO_4 \cdot 2H_2O$ 1.81, and glucose 5.5 mmol/L, pH was adjusted to 7.35 with NaOH 1 mmol/L. Ca^{2+}-free solution contained NaCl 134.8, KCl 4.5, HEPES[(*N*-2-hydroxylethyl) piperazine-*N*'-(2-ethanesulfonic Acid)] 10, $MgCl_2 \cdot 6H_2O$ 1 , glucose 10 mmol/L, pH was adjusted to 7.40 with Tris. The pH of Kraft-Bruhe solution cotainning egtazic acid 0.5, HEPES 10, $MgCl_2 \cdot 6H_2O$ 3 , KCl 50, glucose 10, *L*-glutamata 50, Taurine 20, and KH_2PO_4 20 mmol/L, was adjusted to 7.40 with KOH 1 mmol/L. The isosmotic solution contained CsCl 85, $CaCl_2 \cdot 2H_2O$ 2, glucose 5, HEPES 10, and sucrose 100 mmol/L, pH was adjusted to 7.40 with Tris. The pipette solution contained CsCl 135, Na_2ATP 3, $MgCl_2 \cdot 6H_2O$ 3, *di-tris*-creatine phosphate 2.5, disodium-creatine phosphate 2.5, HEPES 5, and egtazic acid 0.5 mmol/L, pH was adjusted to 7.30 with Tris. Carbachol (CCh) was prepared as aqueous stock solution 10 mmol/L, and AA, LA, and OA were seperately prepared as 1 mmol/L. Indomethacin and H-7 were prepared as 1 mmol/L and 10 mmol/L, respectively.

Data analysis This experiment is consubstantiality comparision. Control is the current before perfused with fatty acids. All values were expressed as mean±SD. Statistical significance was evaluated by *t*-test.

RESULTS

Effect of fatty acids on I_{CCh} Under the whole-cell configuration, the membrane potential was clamped at -20.0 mV, and I_{CCh} was elicited by carbachol 50 μmol/L. AA, an unsaturated fatty acid (with 4 double bonds) significantly inhibited I_{CCh} in a dose-dependant manner. AA inhibited I_{CCh} to 46 %±8 %, 23 %±5 %, and 3.8 %±0.9 % at 1, 3, and 5 μmol/L, respectively

(Fig 1). Another unsaturated fatty acid LA (with 2 double bonds) also inhibited I_{CCh} to 69 %±10 %, 35 %±5 %, and 7.4 %±1.2 % at the concentration of 1, 5, and 10 μmol/L, respectively (Fig 2).

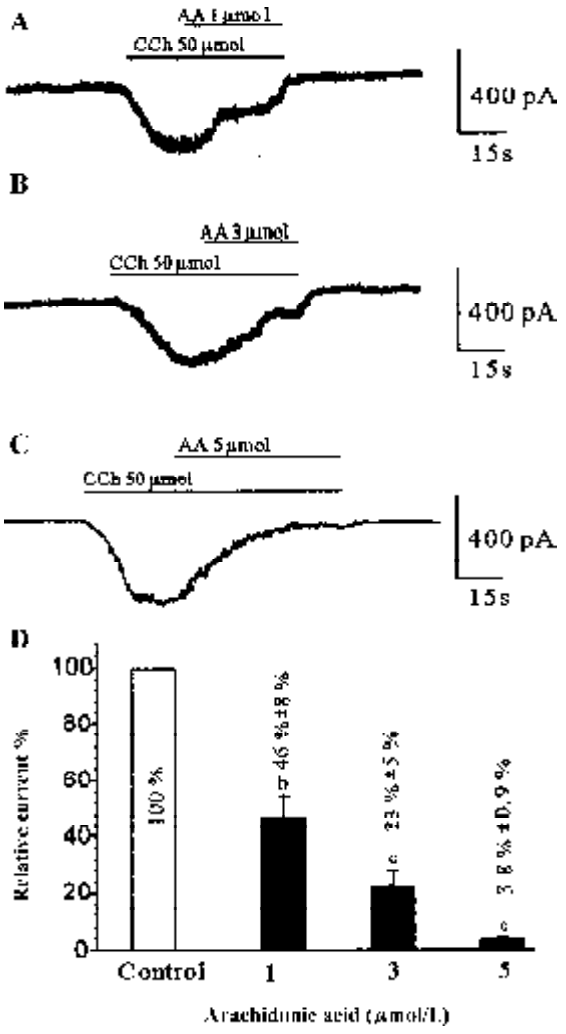

Fig 1. Effect of arachidonic acid on I_{CCh}. A, B, and C show representative current traces which represent inhibitory effects of AA on I_{CCh} in different concentrations, respectively. D shows concentration-dependant inhibition by AA on I_{CCh}. n=6. Mean±SD. ^bP<0.05 *vs* control. Relative current=I_{CCh}/$I_{CCh\ (control)}$×100 %.

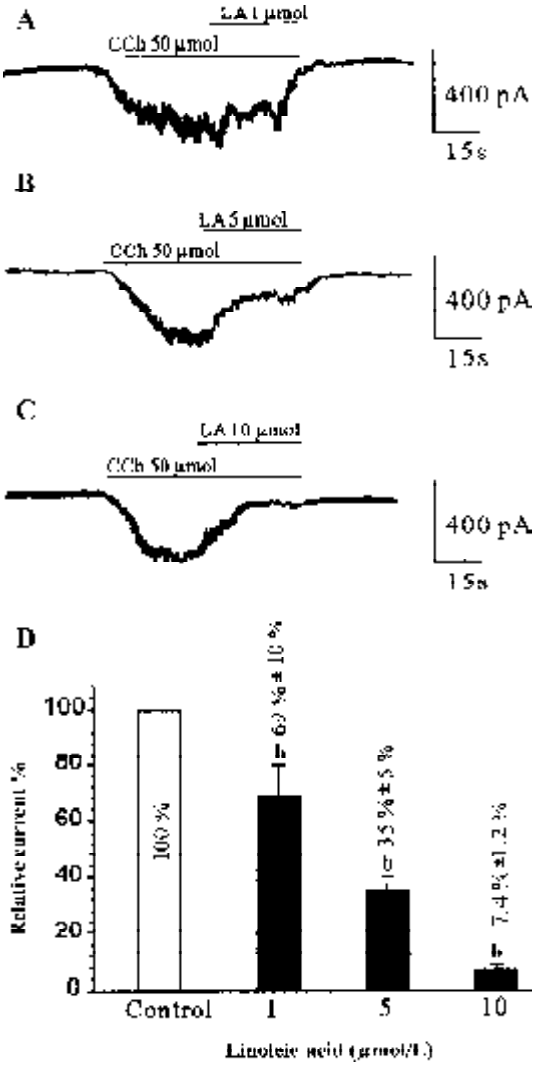

Fig 2. Effect of linoleic acid on I_{CCh}. A, B, and C show representative current traces which represent inhibitory effects of LA on I_{CCh} in different concentrations respectively. D shows dose-dependant inhibition by LA of I_{CCh}. n=6. Mean±SD. ^bP<0.05 *vs* control.

Comparison of the effects among different unsaturated fatty acids on I_{CCh} To determine the inhibitory potency of unsaturated fatty acids, the effects of different unsaturated fatty acids on I_{CCh} was compared. Under the whole-cell configuration, the same concertration (5 μmol/L) of AA, LA, and OA (with one double bond) inhibited I_{CCh} to 3.8 %±0.9 %, 35 %±5 %, and 67 %±9 %, respectively (Fig 3). Both of them intercomparison, the inhibitory potency sequence was AA (C20: 4, *cis*-5, 8, 11, 14)>LA (C18: 2, *cis*-9, 12)> OA (C18: 1, *cis*-9). The inhibitory potency of unsaturated fatty acids was in accordance with the number of double bonds in the fatty acid chain.

Effect of unsaturated fatty acids on I_{CCh} after pretreatment with H-7 and indomethacin To determine whether the inhibitory potency of unsaturated fatty acids on I_{CCh} is direct or indirect, the effect of AA on I_{CCh} was observed, after pretreatment with indomethacin and H-7 for about 10-15 min. In the presence of H-7 (protein phosphorylation C inhibitor) 100 μmol/L and indomethacin (cyclooxygenase inhibitor) 10 μmol/L, AA still inhibited I_{CCh} to 5.5 %±0.7 % and 3.0 %± 1.0 %, respectively (Fig 4). Compared with the effect

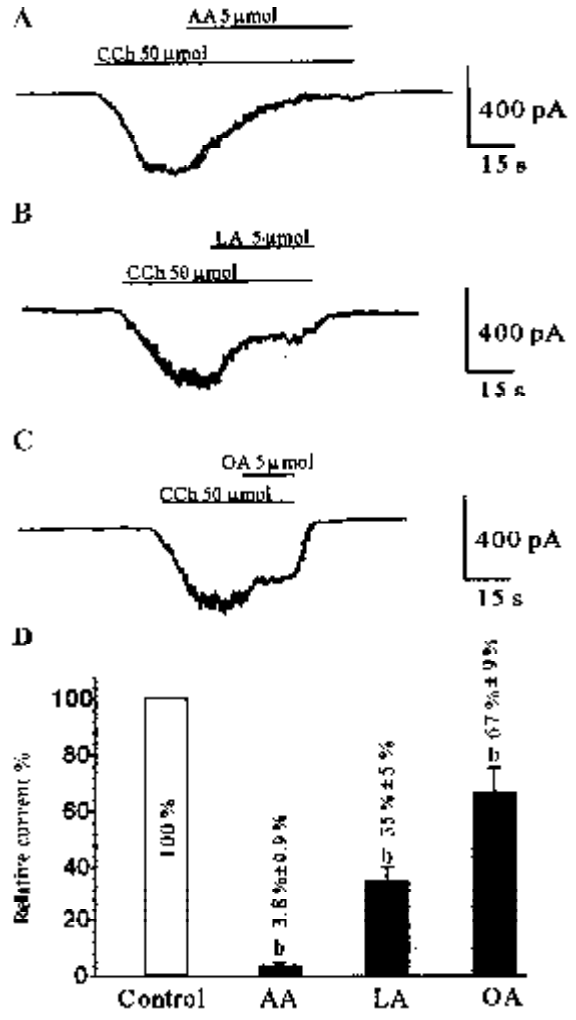

Fig 3. Comparison of the effects among different unsaturated fatty acids 5 m mol/L on I_{CCh}. A, B, and C showed representative current traces which represented one cell in each group, respectively. D showed the inhibitory effect of different unsaturated fatty acids on I_{CCh}. n=8. Mean±SD. ^{b}P<0.05 *vs* control.

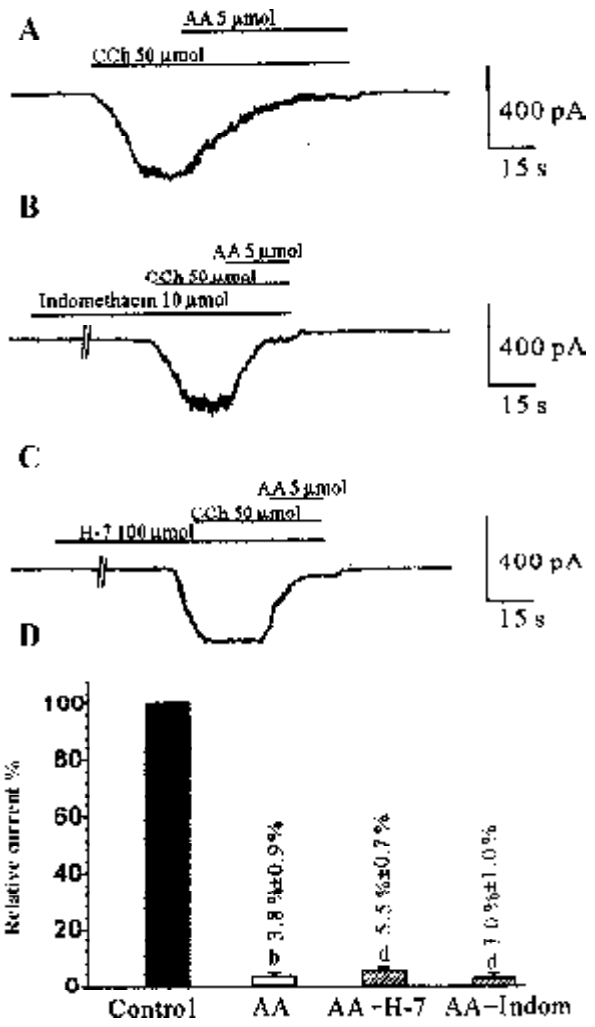

Fig 4. Effect of unsaturated fatty acids on I_{CCh} after pretreatment with H-7 and indomethacin. A, B, and C show representative current traces which represent one cell in each group, respectively. D shows the inhibitory effect of different unsaturated fatty acids on I_{CCh} after H-7 and indomethacin were pretreated. n=8. Mean±SD. ^{b}P<0.05 *vs* control. ^{d}P>0.05 *vs* AA.

of AA on I_{CCh}, there was no significant difference between the inhibitory potency before and after pretreatment (P>0.05).

DISCUSSION

The major findings from this investigation were as follows: 1) I_{CCh} was inhibited by unsaturated fatty acids in a concentration-dependant manner; 2) There was a significant correlation between the degree of *cis* unsaturation and the inhibitory potency on I_{CCh}; 3) The metabolism and PKC pathways were not involved in the inhibitory effect of unsaturated fatty acid on Icch. In our previous study, we got the similar results in calcium current[11] and Cl^- current[12]. Previous investigators also observed an inhibitory effect of unsaturated fatty acid on other ion channels and suggested that the fatty acid might elicit its effects by interacting with the channel itself, or by altering the lipid bilayer in guinea pig vas differens smooth muscle[14].

The regulation of fatty acids on ion channels is either direct or indirect. Indirect effects require the metobalite transformation of fatty acids to biologically active oxygen-containing metabolites[4,15]. Whereas the direct actions appear to arise from an interaction between the fatty acid and ion channel protein or an associated site within the cell membrane[6,16]. The effects of fatty acids on ion channel appear to be ion channel

specific. For example, a number of ion channels, including $GABA_A$ channels, only responded (activation and inhibition) to fatty acids that have *cis*- double bonds, whereas others were equally effected by *cis*-, *trans*-, or saturated fatty acids[17-19]. For some ion channels, there is a nonspecific effect of fatty acids, whether it is by alterations in membrane fluidity or via other mechanisms[6,20]. AA inhibited $I_{(Ca,L)}$ via a mechanism which involved in part stimulation of protein phosphatase activity[21]. AA directly and non-selectly inhibited both K^+ and Ca^{2+} currents in isolated type I cells of the rat carotid body[10]. In present study, although the machanism of this inhibitory effect of AA on Icch was still unclear, we suppose that the effect of AA on Icch was a direct one as we found that indomethacin (a cyclooxygenase inhibitor) and H-7 (protein phosphorylation C inhibitor) did not block the effect of unsaturated fatty acid on I_{CCh}. The direct action of unsaturated fatty acids on membrane phospholipid may imply an effect commanly reffered to as membrane fluidity and this possibility is strengthened by the observed results that the potencies of these inhibitory effects were related to the number of double bonds in the fatty acid chain.

In summary, AA and other unsaturated fatty acids might directly inhibit muscarinic current. The present result suggested that AA and other fatty acid levels in cell membrane may merely affect the sensitivity of smooth muscle membrane under the physiological or experimental stimuli, and modulation of unsaturated fatty acid on ion channel activities may play an important role in physiolosicol condition.

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