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Vasodilator Action of Docosahexaenoic Acid (DHA) in Human Coronary Arteries *In Vitro*

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Abstract

Coronary arterial tissues obtained from mammalian hearts are known to develop spontaneous phasic contractions. The aim of the present study was to investigate the vasodilatory effects of docosahexaenoic acid (DHA) on the rhythmic contractions of isolated human coronary arterial (HCA) preparations obtained from the recipient hearts of patients undergoing cardiac transplantation. Results from 8 hearts show that: (i) most HCA tissues displayed spontaneous rhythmic phasic contractions with a cycle length around 10 min in the absence or presence of $PGF_{2\alpha}$ or elevated $[K^+]_o$ (20 mM); (ii) the rhythmic activity could be suppressed by a free fatty acid DHA (30 μM); (iii) high $[K^+]_o$ (20 and 80 mM) could induce sustained tonic contraction in addition to phasic contractions in HCA tissues, the tonic contraction could be antagonized by L-type Ca^{2+} channel blockers or by DHA (depending on $[K^+]_o$); (iv) a digitalis substance ouabain also could induce tonic contraction and suppress phasic contraction; (v) in isolated HCA vascular smooth muscle cells, DHA increased the magnitude of outward voltage-gated K^+ (I_{KV}) currents and the inwardly rectifying I_{K1} currents. Enhancement of K^+ currents could be related to vasorelaxation induced by DHA in HCA preparations. Further studies on the effects of DHA on various ionic currents and intracellular Ca^{2+} transient are needed to clarify the Ca^{2+}-dependent and the Ca^{2+}-independent actions of DHA in HCA.

Key Words: docosahexaenoic acid (DHA), explanted human hearts, coronary artery smooth muscle, rapid A-type K^+ currents, inwardly rectifying K^+ currents (I_{K1}), voltage-gated K^+ currents (I_{KV}), rhythmic phasic contraction, tonic contraction

Introduction

Isolated human coronary arteries (HCA) are known to develop spontaneous phasic contraction (3, 11). There is experimental evidence for increased synthesis of $PGF_{2\alpha}$-receptors in vascular smooth muscle (VSM) cells that may mediate smooth muscle proliferation (12) and contraction (13). Current evidence indicates that VSM cells express different types of K^+ channels, Ca^{2+} channels, chloride channels, store-operated Ca^{2+} channels and stretch-activated cation channels (6). The interference of both TXA_2 and PGI_2 with K^+-channels in coronary arteries may have important implications for coronary vasospasm and ischemia-related cardiac hypoperfusion.

Some of the effects of Ca^{2+} antagonists on arterial

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tone may be the consequence of antagonism at vascular smooth muscle site which Ca^{2+} is released or interacted, rather than of block of Ca^{2+} entry through membrane L channels (7, 13). $PGF_{2\alpha}$-induced rhythmicity in human coronary arteries could also be inhibited by ouabain, or alterations in K^+ concentrations and by adenosine analogs (11).

Docosahexaenoic acid (DHA) has been shown to induce vasorelaxation in spontaneously hypertensive rats (4). DHA's vaso-relaxant actions in aortic rings of hypertensive rats are independent of endothelium-derived nitric oxide; however, at DHA concentrations ≥ 30 μmol/l, vasodilatory prostanoids that activate ATP-sensitive K^+ channels (K_{ATP}) may be involved. At lower concentrations, DHA-induced relaxation appears to be attributed to modulation of intracellular Ca^{2+} release and L-type Ca^{2+} channels in VSM cells.

The aim of the present study was to investigate the vasodilatory effects of DHA on the rhythmic contractions of isolated HCA preparations obtained from the recipient hearts of cardiac transplantation. Also attempt was made to explore the underlying cellular mechanisms responsible for the inhibitory actions of DHA on phasic contraction of HCA induced by prostanoids ($PGF_{2\alpha}$) and alterations in $[K^+]_o$ concentrations.

Materials and Methods

In Vitro Experiments on Isolated Human Coronary Arterial Tissues

Human left anterior descending or circumflex coronary arteries (HCA) were taken from the failing hearts of 6 male and 2 female patients immediately after operation for cardiac transplantation. Patients with a mean age of 51 years (ranged from 23-65 years) were suffering dilated (n = 7) or ischemic cardiomyopathy (n = 1). In addition to cardiomyopathy, 2 of these 8 transplant recipients also had type 2 diabetes mellitus. Institutional rules for the protection of human subjects were observed. Before surgery, informed consent was obtained. Segments of the HCA were dissected out free of connective tissue and cut into rings of approximately 3 mm in diameter and 4-5 mm long as described previously for dog coronary arterial preparations (8). The ring preparation was then cut longitudinally, and one end was pinned to the bottom of a tissue bath. The other end was connected with a silk thread to a stainless steal bar attached to a Grass FT03 transducer connected to a Gould TA6000 Recorder. The HCA preparation was superfused with an oxygenated physiological salt solution (Tyrode solution) and stretched with an initial resting force of 1 gram for one hour before experiment. The composition of normal Tyrode solution in mM was: NaCl 137; KCl 4; $MgCl_2$ 0.5; $NaHPO_4$ 0.5; $NaHCO_3$ 15; $CaCl_2$ 2.7 and dextrose 5.5. The solution was saturated with a gas mixture of 97% O_2 and 3% CO_2, yielding a pH value around 7.4 at 37°C. In high $[K^+]_o$ solution, $[K^+]_o$ was increased to either 20 or 80 mM (osmolarity was maintained by reducing NaCl from 137 to 82 mM).

HCA Smooth Muscle Cells and Voltage-Clamp Study

A modified explant method was used for isolation of VSM from HCA preparation (10). In brief, the arterial tissue was washed in cold phosphate-buffered saline and the endothelia were stripped. The tissue was minced into 4 mm^2 pieces, transferred onto culture dishes and allowed to adhere. Then, the 1:1 mixture of Dulbecco's modified Eagle medium and Ham's F-12 medium supplemented with penicillin (100 U/ml) and streptomycin (100 μg/ml), 10% fetal bovine serum (FBS) were added and incubated in a 5% CO_2 incubator at 37°C. After confluency, the cells were harvested with 0.25% trypsin and subcultured. Cells of passages 3-6 were used for this study. In addition, healthy CASMC were purchased from Cell Applications Inc (San Diego, CA, USA) and maintained in culture with minimum essential medium supplemented with 10% fetal bovine serum.

Single suction pipette whole-cell voltage-clamp techniques were used by means of an Axopatch 2008 amplifier (Axon Instruments, Foster City, CA, USA). To record ionic currents of VSM cells, we followed the methods of Gollasch *et al*. (5) with modification. The external solution contained (in mM): NaCl 137, $MgCl_2$ 0.5, $CaCl_2$ 1.8, KCl 5.4, glucose 11, HEPES 10 and $CdCl_2$ 200 μM, adjusted to pH 7.4 with 1 N NaOH. The pipette solution contained (in mM): KCl 20, potassium aspartate 110, $MgCl_2$ 1, EGTA 0.5, Na_2-phosphocreatine 5, Mg_2ATP 5, LiGTP 0.1, HEPES 10, adjusted to pH 7.2 with 1 N KOH. Depolarization-induced outward K^+ currents (I_{KV}) were elicited from a holding potential of -80 mV. Depolarizing pulses of 0.3 s duration with test potentials ranging from -50 to 80 mV in 10 mV steps were used to activate I_{KV} at 35°C. Inwardly rectifying K^+ currents (I_{K1}) were elicited from a holding potential of -40 mV. Test potentials ranging from -20 to -120 mV in 10 mV steps for 1 s were used for I_{K1} measurement. In our experiments for K^+ currents determination, the CASMCs from the same dish were incubated separately in one of the two media for 4 h: control solution and solution containing 10 μM DHA.

Chemicals

DHA (docosahexaenoic acid), $PGF_{2\alpha}$, diltiazem, nifedipine, ouabain and other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA). DHA was dissolved in ethanol as a 0.1 M stock

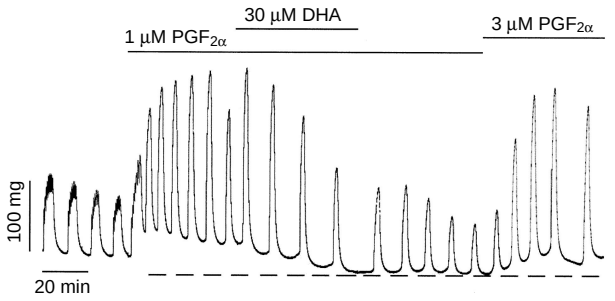

Fig. 1. Effects of DHA on rhythmic contractions of a HCA preparation superfused in 37°C Tyrode solution. $PGF_{2\alpha}$ (1 μM) was administered to enhance the phasic contractions and then DHA (30 μM) was added on top to depress the contractions. Actions of DHA was not reversible after 50 min of washout in 1 μM $PGF_{2\alpha}$ solution/near the end of tracing, the concentration of $PGF_{2\alpha}$ was increased to 3 μM to reverse the depressant action of DHA. Broken line underneath the traces indicates level of resting tension.

solution and stored in a -20°C freezer until use.

Statistical Analysis

Data are presented as means ± S.E.M. Statistical analyses were conducted by using Student's *t* test, and a $P < 0.05$ was considered significant.

Results

Effects of DHA and $PGF_{2\alpha}$ on Phasic Contraction

As shown in Fig. 1, a HCA preparation generated rhythmic contractions with a cycle length around 10 min when superfused in normal Tyrode solution at 37°C. $PGF_{2\alpha}$ at a concentration of 1 μM increased the amplitude of both the maximum and the minimum developed tension and shortened the spontaneous cycle length to 8 min per cycle in 24 min. Addition of 30 μM DHA to the perfusate significantly prolonged the cycle length to 18 min and shifted the tension (both maximum and minimum) downward. Removal of the DHA from the superfusate quickly returned the cycle length to 10 min per cycle but the contractile tension remained depressed until a higher concentration of $PGF_{2\alpha}$ (3 μM) was added.

Similar spontaneous phasic contractions were observed in 6 of 20 HCA preparations tested in normal Tyrode solution. In 3 of 5 additional HCA preparations, the arterial preparation remained silent until 1 μM $PGF_{2\alpha}$ was added. In these 5 preparations, $PGF_{2\alpha}$ increased the maximum contractile tension from 24 ± 21 to 181 ± 49 mg and minimum tension from 6 ± 5 to 33 ± 9 mg. DHA (30 μM) on top of $PGF_{2\alpha}$ reduced the maximum contractile tension to 136 ± 45 mg (-27 ± 8%) and depressed the minimum tension further to 18 ± 7 mg (-43 ± 11%).

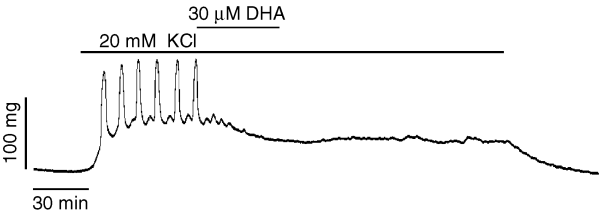

Fig. 2. Phasic contractions induced by 20 mM KCl and their suppression by 30 μM DHA. The HCA preparation was silent in 4 mM KCl Tyrode solution. Increasing $[K^+]_o$ from 4 to 20 mM induced rhythmic contractions at a cycle length of 10 min which were subsequently suppressed by DHA.

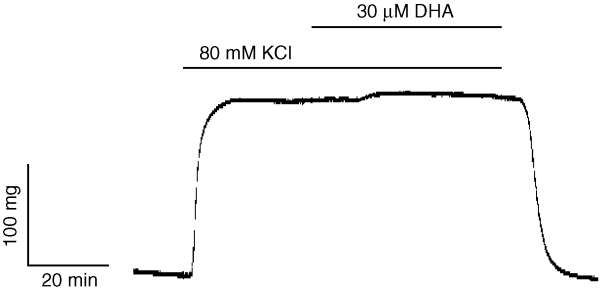

Fig. 3. Higher $[K^+]_o$ (80 mM) induced tonic contraction without phasic activities. A HCA preparation was silent in normal Tyrode solution at the beginning of the tracing. Increasing $[K^+]_o$ from 4 to 80 mM induced a sustained tonic contraction which was barely changed by addition of 30 μM DHA.

Another method was also used to induce phasic contractions in silent HCA preparations. As shown in Fig. 2, a HCA preparation was silent in normal Tyrode solution. When $[K^+]_o$ was increased from 4 to 20 mM, rhythmic contractions occurred at a cycle length of 10 min. Addition of DHA (30 μM) immediately suppressed the rhythms and shifted the minimal tension to a lower value. The suppressive effect was not reversible even after washout of the drug for 120 min.

Effects of DHA on Tonic Contraction Induced by High $[K^+]_o$

In the superfusate containing a higher concentration of KCl (80 mM), the HCA preparations develop tonic contraction without phasic activity as illustrated in Fig. 3. DHA (30 μM) barely changed the tonic tension developed in 80 mM KCl. In 3 HCA preparations, the tonic tension in the absence and presence of DHA were similar (121 ± 49 mg *vs.* 122 ± 46 mg, $P > 0.05$).

In one HCA preparation, phasic contractions developed spontaneously in normal Tyrode with a cycle

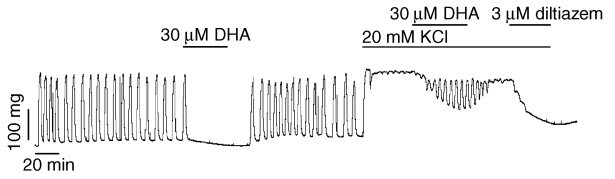

Fig. 4. Relaxant effect of DHA on tonic contracture induced by 20 mM KCl in a HCA preparation. Traces began with the spontaneous rhythmic contractions in normal $[K^+]_o$ Tyrode solution. DHA (30 μM) reversibly suppressed the phasic contractions (left side). Increasing $[K^+]_o$ from 4 to 20 mM induced tonic contraction which was relaxed intermittently and reversibly by 30 μM DHA. In contrast, 3 μM diltiazem induced a complete relaxation in 20 mM $[K^+]_o$ solution (near right margin).

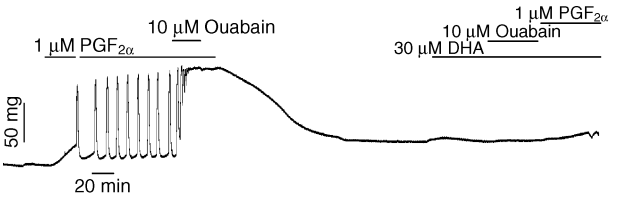

Fig. 5. Tonic contraction induced by 10 μM ouabain in a HCA preparation. In normal Tyrode solution, 1 μM $PGF_{2\alpha}$ induced phasic contractions at a cycle length of 10 min. Addition of 10 μM ouabain on top of $PGF_{2\alpha}$ induced a sustained contracture which was reversible but very slowly after washout. DHA (30 μM) prevented contracture even in the presence of 10 μM ouabain plus 1 μM $PGF_{2\alpha}$ (traces near right margin).

length of 7.35 min (Fig. 4). Addition of 30 μM DHA immediately suppressed the rhythmic contractions and shifted the minimum tension downward. The changes in tension were quickly reversible after washout of DHA for 22 min. Increasing $[K^+]_o$ from 4 to 20 mM induced a sustained contraction to a level similar to the maximum phasic tension in the previous DHA exposure in 4 mM $[K^+]_o$ solution. The tension of preparation then oscillated slightly but failed to relax until DHA was added 60 min later. During DHA exposure in 20 mM $[K^+]_o$, the relaxation become deeper and deeper with a rhythmic cycle length of around 5 min. Thus, in tonic contraction induced by 20 mM $[K^+]_o$, DHA was still able to induce relaxation presumably through a reduction of cellular $[Ca^{2+}]_i$. This assumption was supported by the fact that, after washout of DHA, addition of 3 μM diltiazem in 20 mM $[K^+]_o$ superfusate caused complete relaxation in 45 min (right panel in Fig. 4).

Effects of DHA on Tonic Contraction Induced by Na^+/K^+ Pump Inhibition

In addition to high $[K^+]_o$, tonic contraction could also be induced by a digitalis substance, 10 μM ouabain. As shown in a silent HCA preparation perfused in normal Tyrode solution, addition of 1 μM $PGF_{2\alpha}$ induced phasic contractions with a cycle length of around 10 min. Ouabain (10 μM) on top of $PGF_{2\alpha}$ inhibited relaxation and induced a sustained tonic contraction in 16 min (Fig. 5). The tonic contraction was reversible after a prolonged washout period (130 min) in normal (4 mM $[K^+]_o$) Tyrode. Thereafter, addition of 30 μM DHA plus ouabain induced only small magnitude tonic contraction without phasic activity.

Effects of High $[K^+]_o$ in the Actions of DHA

In most HCA preparations, phasic contractions occurred in 4 mM $[K^+]_o$ with or without $PGF_{2\alpha}$. However, as shown in an example illustrated in Fig. 4, increasing $[K^+]_o$ from 4 to 20 mM could turn phasic contraction into tonic contraction which could be changed into rhythmic phasic activities by adding DHA (but not by diltiazem), suggesting the complex actions of DHA on both sarcoplasmic reticulum and sarcolemma in HCA. In tonic contraction induced by 80 mM $[K^+]_o$, DHA barely changed the tension as illustrated in Fig. 3. Further studies on the effects of DHA on intracellular Ca^{2+} transient are required to clarify the Ca^{2+}-dependent and the Ca^{2+}-independent actions of DHA in HCA.

Effects of DHA on K^+ Currents

In smooth muscle cells isolated enzymatically from HCA preparations obtained at cardiac transplan-tation, depolarizing pulses from a holding potential of -80 mV to test potentials ranging from -50 mV to +80 mV in 10 mV steps for 300 ms could induce outward K^+ currents (I_{KV}) as illustrated in Fig. 6. The HCA smooth muscle (HCASM) cells were incubated in 3 different media (DHA-free, solution containing 1 μM and 10 μM DHA) for 4 h. In addition to the steady-state I_{KV}, there was also rapid transient outward K^+ currents (rapid I_A, see ref. 1) early on depolarization to test potentials ranging from 20 to 80 mV. The higher concentration of DHA (10 μM) appeared to increase markedly the I_{KV} at higher depolarizing potentials (> 40 mV) (Fig. 6C) but due to the large variation in the data obtained from transplant recipients (*e.g.* control mean ± S.E.M. = 8.7 ± 3.4 pA/pF, n = 19 *vs*. 10 μM DHA 16.5 ± 4.8 pA/pF, n = 11, at test potential of +80 mV) the difference between groups was not statistically significant ($P > 0.05$). One possible reason for the lack of significant effect of DHA on I_{KV} could be due to variations in the electrophysiological properties of the HCASM cells obtained from recipients of cardiac transplantation.

In healthy HCASM cells purchased from Cell

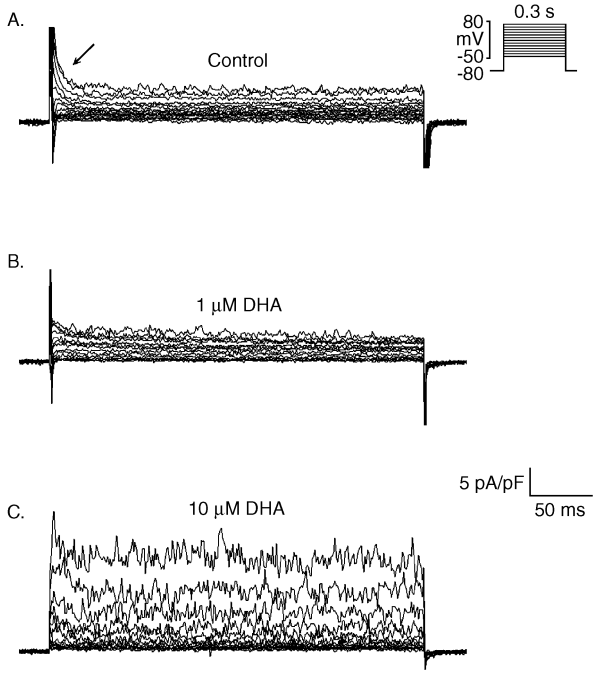

Fig. 6. Effects of DHA on outward voltage-gated K^+ currents (I_{KV}) in smooth muscle cells isolated from HCASM preparations obtained from the recipient hearts. For electrophysiological studies, isolated cells were incubated in one of the 3 media at 37°C without (Control) or with 1 μM DHA (panel B) and 10 μM DHA (panel C) for 4 hours. The I_{KV} currents were measured at 35°C with the voltage-clamp protocol shown at right upper corner. I_{KV} current traces were induced on depolarization from a holding potential of -80 mV to test potentials ranging from -50 up to 80 mV in 10 mV steps in cell cultured without (Control, panel A), with 1 μM DHA (panel B) and 10 μM DHA (panel C). Note in panel A the presence of I_A current (indicated by an arrow) early on depolarization.

Application Inc., DHA increased consistently the I_{KV} over the potential range from -50 to +80 mV (Fig. 7, C and D). There were also I_{K1} currents on repolarization as illustrated in Fig. 7A. The I_{K1} currents showed inward rectification at potentials more negative than -40 mV but outward currents at potentials range more positive than -30 mV was very small when present. DHA (10 μM) increased magnitude of the inward rectifier I_{K1} as shown in the current-voltage relationship (Fig. 7B).

Discussion

The present experiments in human coronary arterial tissues (HCA) show that: (i) most HCA tissues displayed spontaneous phasic contractions with a cycle length around 10 min in the absence or presence of $PGF_{2\alpha}$ or elevated $[K^+]_o$ (20 mM); (ii) the rhythmic activity could be suppressed by a free fatty acid DHA (30 μM) or by L-type Ca^{2+} channel blockers (nifedipine or diltiazem); (iii) high $[K^+]_o$ (20 and 80 mM) could also induce sustained tonic contraction in addition to phasic contractions in HCA tissues, the tonic contraction could be or could not be antagonized by DHA, depending on $[K^+]_o$; (iv) a digitalis substance ouabain also could induce tonic contraction and suppress phasic contraction; (v) in cultured vascular smooth cells obtained from healthy HCA, DHA increased the magnitude of voltage-gated K^+ (I_{KV}) and I_{K1} currents which could be related to vaso-relaxation induced by DHA in HCA preparations.

Phasic vs. Tonic Contraction

In most HCA preparations, phasic contractions occurred in 4 mM $[K^+]_o$ with or without $PGF_{2\alpha}$. However, as shown in an example illustrated in Fig. 4, increasing $[K^+]_o$ from 4 to 20 mM could turn phasic contraction into tonic contraction which could be changed into rhythmic phasic activities by adding DHA (but not by diltiazem), suggesting the complex actions of DHA on both sarcoplasmic reticulum and sarcolemma in HCA. By means of intracellular Ca^{2+} transient ($[Ca^{2+}]_i$) measurement, it has been shown that vasopressin (100 nM) elicited an initial peak $[Ca^{2+}]_i$ followed by a sustained phase due to Ca^{2+} entry (2) in rat aortic smooth muscle cell lines. Nifedipine (1 μM) partly inhibited the sustained phase, but La^{3+} completely abolished them. These results are in agreement with the concept that the phasic contraction of arteries is due to a release of Ca^{2+} from the cellular stores while the tonic contraction is intimately related to trans-sarcolemmal Ca^{2+} influx. Eicosapentaenoic acid (EPA), a n – 3 polyunsaturated fatty acid, inhibited $I_{Ca,L}$ and a non-selective cation current thus could exert hypotensive effects in rats (2).

In our study with DHA (also a n-3 polyunsaturated fatty acid), phasic contractions of HCA were markedly depressed but tonic contraction was only barely changed. It is interesting to note that, in HCA, phasic contraction could turn into tonic contraction by increasing $[K^+]_o$ (Fig. 4) or by the use of a Na^+, K^+-ATPase inhibitor ouabain (Fig. 5), suggesting the important role of $[Ca^{2+}]_i$ in determining phasic *vs*. tonic contraction in HCA.

Role of Sarcolemmal L-Type Ca^{2+} Channels in the Actions of DHA

The important role for $I_{Ca,L}$ in the maintenance of tonic contraction was clearly demonstrated in the action of diltiazem shown in Fig. 4. DHA not only inhibited the maximum phasic contraction (since the Ca^{2+} content in stores were reduced as a result of $I_{Ca,L}$ blockade) but also shifted the minimum contrac-tion to a lower tension level (Figs. 1, 2 and 4) as a consequence

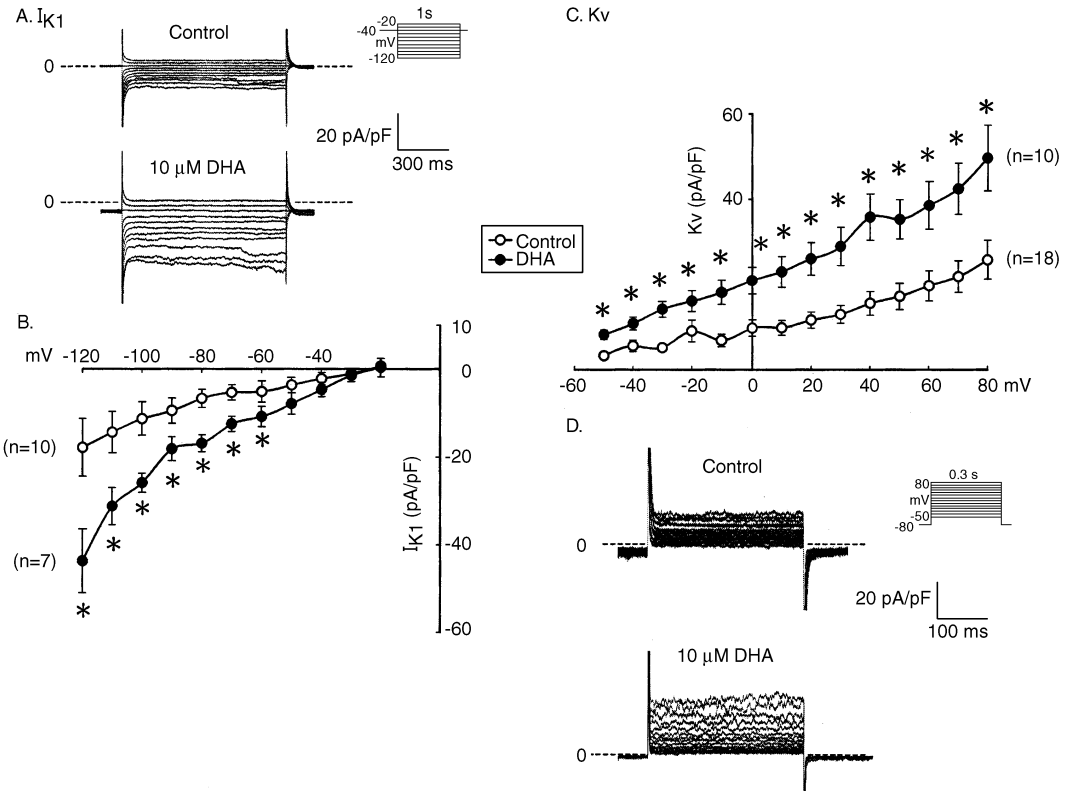

Fig. 7. Effects of DHA on inwardly rectifying K^+ currents (I_{K1}) currents and outward voltage-gated K^+ currents (I_{KV}) in healthy HCASM cells purchased from Cell Applications, Inc., (San Diego, CA, USA). For electrophysiological studies, isolated cells were incubated in one of the 2 media at 37°C without (Control) or with 10 μM DHA for 4 h. The ionic currents were measured at 35°C. Panel A shows examples of I_{K1} measured without (Control) and with 10 μM DHA, from a holding potential of -40 mV to test potentials ranging from -20 down to -120 mV in 10 mV steps. Panel B shows the I_{K1} current-voltage relationship. Panel D shows I_{KV} current traces on depolarization from a holding potential of -80 mV to test potentials ranging from -50 up to 80 mV in 10 mV steps in cell cultured without (Control) and with 10 μM DHA (bottom traces). Broken lines indicate holding currents. Panel C shows the current density of I_{KV} in pA/pF over test potentials ranging from -50 to 80 mV. Numbers of cells from each group are indicated. $*P < 0.05$, significantly different from control value by group comparison.

of a relief from $[Ca^{2+}]_i$ over loading (14). These actions of DHA were reversible after washout of the drug.

Effects of Elevated $[K^+]_o$ in the Actions of DHA

In tonic contraction induced by 80 mM $[K^+]_o$, DHA barely changed the tension as illustrated in Fig. 3. The tonic contraction induced by 20 mM $[K^+]_o$ in 2 preparations (167 ± 45 mg) was not significantly different from the average values in 80 mM $[K^+]_o$ (148 ± 38 mg, n = 4, $P > 0.05$) except that only the tonic contraction induced in 20 mM $[K^+]_o$ could be reduced by DHA (Fig. 4). Further studies on the effects of DHA on intracellular Ca^{2+} transient are required to clarify the Ca^{2+}-dependent and the Ca^{2+}-independent actions of DHA in HCA.

Effects of DHA on K^+ Currents in VSM Cells

Enhancement of outward K^+ currents would shift the maximum diastolic potential to a more negative value and stabilize membrane potential of smooth muscle cells thus induce dilations in arteries. Therefore the increase in I_{KV} induced by DHA in human coronary smooth muscle cells would relax HCASM. In addition, elevation of $[K]_o$ could exert action on inwardly rectifying K^+ channels of HCA smooth muscle cells and induce relaxation as observed in murine cerebral arteries (15) and rabbit coronary arteries (9). Also DHA appeared to block the transient outward I_A currents as suggested by the recording in Fig. 6A. Further experiments are required to clarify the actions of DHA on various ionic currents in HCA.

Acknowledgments

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