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Short communication

Blockade of two voltage-dependent potassium channels, mKv1.1 and mKv1.2, by docosahexaenoic acid

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Abstract

The effects of the polyunsaturated fatty acid, docosahexaenoic acid, were examined on two single cloned potassium channels, mKv1.1 and mKv1.2, stably expressed in Chinese hamster ovary cells using whole-cell patch clamp techniques. Docosahexaenoic acid produced a time- and dose-dependent, reversible block of mKv1.1 and mKv1.2. Interestingly, docosahexaenoic acid increased the rate of activation of mKv1.2 leading to an enhancement of current amplitude at short intervals following activating the voltage step. This phenomenon was not seen in the case of mKv1.1. Intracellular administration of docosahexaenoic acid did not block either type of channel. These findings suggest that docosahexaenoic acid inhibits mKv1.1 and mKv1.2 channels by acting at an extracellular site and by an open-channel blocking mechanism.

Keywords: Polyunsaturated fatty acid; Docosahexaenoic acid; K^+ channel, cloned; Patch clamp, whole-cell

1. Introduction

Recently there has been considerable interest in the effect of polyunsaturated fatty acids on ionic currents. Docosahexaenoic acid (*cis*-4,7,10,13,16,19-docosahexaenoic acid) is a long-chain polyunsaturated fatty acid which is abundant in fish oil and is an essential component of the neuronal cell membranes (Poling et al., 1995a). Docosahexaenoic acid produces a time-dependent block of both the delayed rectifier (I_K) and the early transient current (I_A) in pineal cells (Poling et al., 1995a). Honoré et al. (1994) have also reported a time-dependent block of the cardiac voltage-dependent potassium channel, Kv1.5, by docosahexaenoic acid. Another polyunsaturated fatty acid, arachidonic acid, has been shown to increase the M current (I_M) in bull frog neurones (Bosma et al., 1990; Yu, 1995). In hippocampal neurones both arachidonic acid and its metabolite, leukotriene C_4, appear to mediate the somatostatin-induced augmentation in I_M (Schweitzer et al., 1993) and arachidonic acid augments potassium currents in rat neocortical neurones (Zona et al., 1993). In contrast, cloned mKv1.2 channels are blocked by arachidonic acid (McEvoy et al., 1995).

The present study investigates the effect of docosahexaenoic acid on two cloned voltage-dependent potassium channels, mKv1.1 and mKv1.2, stably expressed in Chinese hamster ovary (CHO) cells. mKv1.1 and mKv1.2 are 98% homologous and have delayed-rectifier-like properties although mKv1.2 has a slower activation rate. Preliminary accounts of some of these findings have already been presented (Garratt et al., 1995; Garratt and Owen, 1996).

2. Materials and methods

2.1. Cell culture

CHO mKv1.1 and mKv1.2 cell lines were supplied by Dr. Bruce Tempel (University of Washington, Seattle, WA, USA). The cell line was generated as described by Bosma et al. (1993). Cells were grown in tissue culture in 75 ml flasks in medium containing 92% RPMI 1640 medium (Sigma), 5% foetal bovine serum, 1% L-glutamine (ICN Flow, High Wycombe, UK), 1% antibiotic solution (Sigma) and 1% geneticin solution (0.3% mg/ml, geneticin sulphate in 10% HEPES-buffered distilled water

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solution). Flasks of cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2 in air. Cells were subsequently plated onto 35 mm Petri dishes (Gibco, UK) at least 2 h prior to electrophysiological recording.

2.2. Electrophysiology

CHO cells were investigated by the whole-cell patch clamp technique. Patch electrodes were pulled from thin-walled borosilicate glass (GC150TF-10, Clark Electromedical Instruments, Reading, UK) using a DMZ Universal Puller (Zeitz Instrumente, Germany) and had a resistance of 5–10 MΩ. Voltage-gated currents were acquired and stored on a Quadra 800 Macintosh computer using an EPC9 amplifier (HEKA Elektronic, Lambrecht, Germany). In Fig. 1A and B currents were elicited by 500 ms depolarising pulses from a holding potential of −90 mV and were sampled at a rate of 500 μs per point and digitally filtered by a low-pass Bessel filter set at 0.7 Hz; in Fig. 2A–C currents were elicited by 9 s depolarising pulses from a holding potential of −90 mV and were sampled at a rate of 1 ms per point and digitally filtered by a low-pass Bessel filter set at 0.3 Hz; in Fig. 2D currents were elicited by a 120 ms pulse from a holding potential of −90 mV and were sampled at a rate of 150 μs per point and digitally filtered by a low-pass Bessel filter set at 2.2 Hz. All currents were capacitance subtracted and leak subtracted on-line with a p/4 subtraction protocol. Measurements of block of the current by docosahexaenoic acid were made from records where there was no further change in the amplitude of the current in the presence of a particular concentration applied.

2.3. Recording solutions

Electrodes were filled with solution which comprised (mM): potassium aspartate, 120; KCl, 20; $MgCl_2$, 1; MgATP, 2; EGTA, 10; HEPES, 10; pH 7.4, adjusted with NaOH. Cells were mounted in a recording chamber and continuously perfused via a micropipette at 3 ml/min with an 'extracellular solution' which comprised (mM): NaCl, 135; $MgCl_2$, 4; KCl, 5; $CaCl_2$, 2; glucose, 25; HEPES, 10; EGTA, 1 pH 7.4 adjusted with NaOH. Drugs were dissolved in the extracellular solution and applied directly to cells via gravity feed. For intracellular administration the drug was dissolved in the patch pipette solution. All experiments were conducted at room temperature (21–24°C). Docosahexaenoic acid and rubidium chloride were obtained from Sigma.

2.4. Data analysis

mKv1.1 and mKv1.2 activity was quantified as the overall charge transfer during the voltage step (Q_{step})

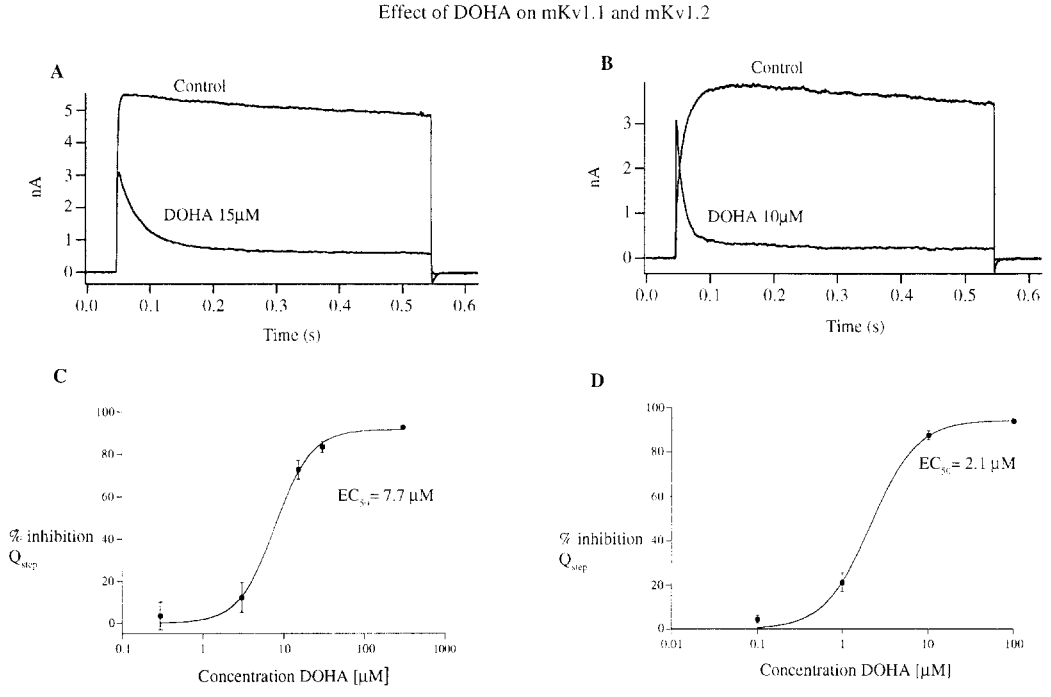

Fig. 1. The effect of extracellular docosahexaenoic acid on both mKv1.1 and mKv1.2 channels stably expressed in CHO cells. (A) A trace showing the mKv1.1 current is elicited with a voltage step from −90 to +70 mV both in control conditions and in the presence of docosahexaenoic acid (15 μM). Docosahexaenoic acid causes a time-dependent block during the voltage step indicating open channel block. (B) A trace showing the mKv1.2 current elicited with a voltage step from −90 to +70 mV both in control conditions and in the presence of docosahexaenoic acid (10 μM). Docosahexaenoic acid causes a progressive block during the voltage. Note the increase in the activation rate. (C) and (D) are dose-response curves of mKv1.1 and mKv1.2 respectively. Inhibition is calculated in terms of % reduction in the charge transfer during the voltage step (Q_{step}).

calculated from the integral of current over the duration of the voltage pulse. Block was expressed as a percentage of the control pre-drug Q_{step}. Time constants of decay (τ_D) were derived from a single exponential fitted to currents using the Pulsefit program (HEKA, Lambrecht, Germany). All data are expressed as means ± S.E.M., with statistical analysis performed using two sample *t*-tests.

3. Results

External administration of docosahexaenoic acid produced a dose-dependent, reversible block of mKv1.1 (EC_{50} = 7.7 μM) (Fig. 1A and C) and mKv1.2 (EC_{50} = 2.1 μM) (Fig. 1B and D). Blockade of mKv1.1 and mKv1.2 channels is also time-dependent, e.g. at +70 mV in the presence of docosahexaenoic acid (15 μM) for mKv1.1 decayed with a time constant (τ_D) of 52.5 ± 4.5 ms. In the case of mKv1.2 docosahexaenoic acid (10 μM) blocked with a τ_D 11.6 ± 0.3 ms. The time constants of the docosahexaenoic acid-induced decay decreased in a concentration-dependent fashion in both cases, e.g. for mKv1.1 τ_D of 68.4 ± 0.5 ms at 10 μM compared with τ_D of 23.7 ± 0.3 ms at 100 μM. Interestingly, docosahexaenoic acid was found to increase the rate of activation (Fig. 1B) of mKv1.2 (0.9 ms compared with the control value of 2.5 ms) such that the current amplitude was in fact greater than control up to 10 ms following the depolarising pulse. Intracellular administration of docosahexaenoic acid (300 μM) did not alter either mKv1.1 or mKv1.2 currents although in the same cells subsequent extracellular administration of docosahexaenoic acid did reduce the current.

By increasing the length of the voltage step to 9 s it was possible to observe slow inactivation of the mKv1.1 and mKv1.2 currents. This current could be fit with two exponentials, τ_{Dslow1} and τ_{Dslow2} (Fig. 2A). Docosahexaenoic acid produced some decrease in τ_{Dslow1} (0.8 ± 0.3 s compared to control of 2.3 ± 0.7 s) and when the two curves were scaled they were shown to cross over (Fig. 2B). The mKv1.2 current elicited under this protocol showed less inactivation across the step than mKv1.1; the block was still time-dependent (Fig. 2C).

The inclusion of rubidium (35 mM substitution for NaCl) increased mKv1.1 tail current amplitude and duration, thus permitting more facile study of tail currents (Fig. 2D). Docosahexaenoic acid decreased the amplitude of the tail current (22 ± 4% of control) and increased the deactivation time constant of the tail current (45 ± 1.8 ms compared to control values of 35 ± 2.5 ms) (Fig. 2D).

4. Discussion

Docosahexaenoic acid produced a time- and dose-dependent blockade of mKv1.1 and mKv1.2 channels and this effect was reversible. Docosahexaenoic acid appears to

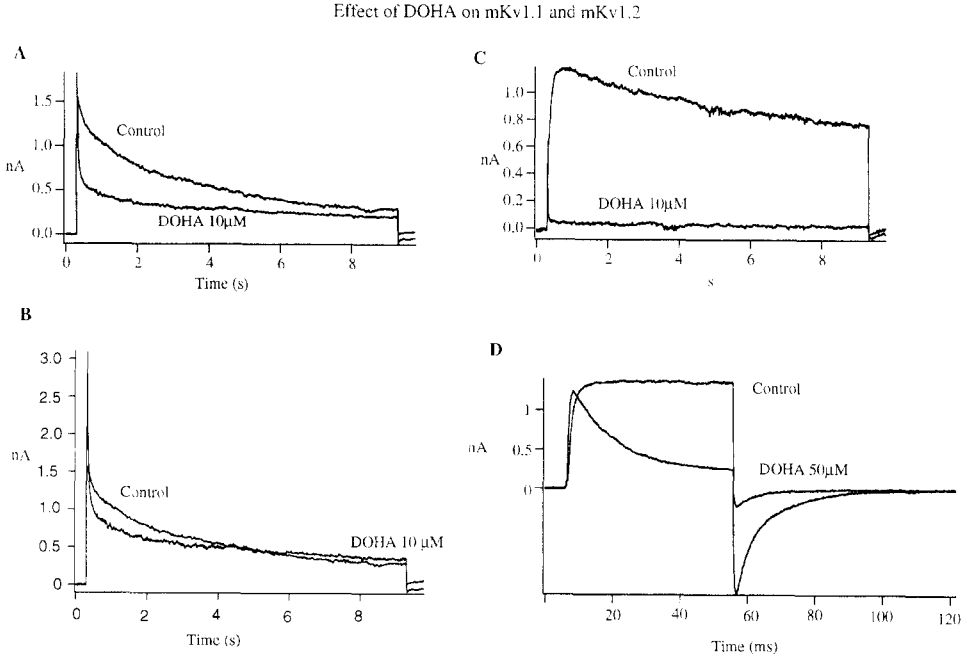

Fig. 2. (A, B and C) The effect of docosahexaenoic acid on the mKv1.1 and mKv1.2 current produced by a longer (9 s) voltage step. (A) slow inactivation of mKv1.1 current elicited by this protocol and the block by docosahexaenoic acid, (B) the same data except that blocked current has been scaled to illustrate the slowing of the slower component. (C) The mKv1.2 currents elicited by this protocol shows less inactivation over the step compared with mKv1.1 and block by docosahexaenoic acid is virtually complete after only 100 ms. (D) Shows the effect of docosahexaenoic acid on the mKv1.1 tail currents which have been slowed by the inclusion of Rb in the extracellular solution. Docosahexaenoic acid decreased the amplitude and increased the time constant of deactivation.

have an open-channel blocking mechanism as shown by the decrease in τ_D with increasing concentration. Also if docosahexaenoic acid was applied for a minute without depolarising the cell then on the first positive voltage step there was only a very small decrease in the amplitude of the current. In order, however, to be completely sure docosahexaenoic acid is blocking open channels single channel studies need to be performed to confirm shortening of openings. This compound was slightly more potent at blocking mKv1.2 compared with mKv1.1 and, interestingly, apparently increased the rate of activation, and this is in agreement with the findings with rKv1.2 stably expressed in CL1023 fibroblasts (Poling et al., 1995a). The latter effect did not appear to be dose related, however, at least at ≥ 100 μM. Another polyunsaturated fatty acid, arachidonic acid, has also been shown to increase the rate of activation of mKv1.2 (McEvoy et al., 1995) and it is possible that a common mechanism underlies this phenomenon. Intracellular administration, via the patch pipette, of docosahexaenoic acid did not alter either the mKv1.1 or mKv1.2 current suggesting that it blocks the channels at an external site; this, too, is in agreement with the findings in the rKv1.2 stably expressed in CL1023 fibroblasts (Poling et al., 1995b). Again, too, this is in common with arachidonic acid (McEvoy et al., 1995) suggesting that this may be a common feature of polyunsaturated fatty acids in general. Docosahexaenoic acid blocks the cardiac voltage-dependent potassium channel, Kv1.5 (Honoré et al., 1994) and the transient outward current in pineal cells (Poling et al., 1995a). The fact that docosahexaenoic acid also blocks mKv1.1 and mKv1.2 suggests that it is a non-selective K^+ channel blocker. Indeed preliminary data indicate that Kv1.4 is sensitive also (Garratt et al., unpublished observation).

By using a longer step in the voltage protocol the effects of docosahexaenoic acid on slow inactivation can be examined. Docosahexaenoic acid was found to produce a crossover of the mKv1.1 current and this would support the other findings of this study to suggest that docosahexaenoic acid is acting externally.

This study in agreement with several others that have shown that docosahexaenoic acid is a potent blocker of two members of the Kv1 channel family, mKv1.1 and mKv1.2. In view of the broad spectrum of activity of docosahexaenoic it would be tempting to speculate that dietary docosahexaenoic acid may modulate excitable cells to some degree.

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